This is a digital copy of a book that was preserved for generations on library shelves before it was carefully scanned by Google as part of a project to make the world's books discoverable online.

It has survived long enough for the copyright to expire and the book to enter the public domain. A public domain book is one that was never subject to copyright or whose legal copyright term has expired. Whether a book is in the public domain may vary country to country. Public domain books are our gateways to the past, representing a wealth of history, culture and knowledge that's often difficult to discover.

Marks, notations and other marginalia present in the original volume will appear in this file - a reminder of this book's long journey from the publisher to a library and finally to you.

Usage guidelines

Google is proud to partner with libraries to digitize public domain materials and make them widely accessible. Public domain books belong to the public and we are merely their custodians. Nevertheless, this work is expensive, so in order to keep providing this resource, we have taken steps to prevent abuse by commercial parties, including placing technical restrictions on automated querying.

We also ask that you:

+ *Make non-commercial use of the files* We designed Google Book Search for use by individuals, and we request that you use these files for personal, non-commercial purposes.

+ *Refrain from automated querying* Do not send automated queries of any sort to Google's system: If you are conducting research on machine translation, optical character recognition or other areas where access to a large amount of text is helpful, please contact us. We encourage the use of public domain materials for these purposes and may be able to help.

+ *Maintain attribution* The Google "watermark" you see on each file is essential for informing people about this project and helping them find additional materials through Google Book Search. Please do not remove it.

+ *Keep it legal* Whatever your use, remember that you are responsible for ensuring that what you are doing is legal. Do not assume that just because we believe a book is in the public domain for users in the United States, that the work is also in the public domain for users in other countries. Whether a book is still in copyright varies from country to country, and we can't offer guidance on whether any specific use of any specific book is allowed. Please do not assume that a book's appearance in Google Book Search means it can be used in any manner anywhere in the world. Copyright infringement liability can be quite severe.

About Google Book Search

Google's mission is to organize the world's information and to make it universally accessible and useful. Google Book Search helps readers discover the world's books while helping authors and publishers reach new audiences. You can search through the full text of this book on the web at `http://books.google.com/`

UNIVERSITY OF WISCONSIN LIBRARY

4th fl
Case 16

5-A-6

DEPARTMENT OF MARINE BIOLOGY
OF
THE CARNEGIE INSTITUTION OF WASHINGTON
ALFRED G. MAYER, DIRECTOR

PAPERS
FROM THE DEPARTMENT OF MARINE BIOLOGY
OF THE
CARNEGIE INSTITUTION OF WASHINGTON

VOLUME XI

WASHINGTON, D. C.
PUBLISHED BY THE CARNEGIE INSTITUTION OF WASHINGTON
1917

CARNEGIE INSTITUTION OF WASHINGTON

PUBLICATION No. 251

es of this Book
e first issued
JUL 24 1917

PRESS OF GIBSON BROTHERS, INC.
WASHINGTON, D. C.

212968
SEP 10 1917
~~MXL
.C21
11~~
.7C214
11

CONTENTS.

PAGE.
I. *Nerve-Conduction in Cassiopea xamachana.* By Alfred Goldsborough Mayer. 15 figures 1-20
Methods and Corrections 3
Experiments 9
Summary 18

II. *The Hydrogen-ion Concentration, CO_2 Tension, and CO_2 Content of Sea-Water.* By J. F. McClendon, C. C. Gault, and S. Mulholland. 24 figures 21-69
Definitions and Abbreviations 23
Results applied to Oceanography and Measurement of Respiration-rate of Marine Organisms 27
Calibration of an Improved Indicator Method for use on Shipboard and in Poorly Equipped Laboratories 42
New Apparatus for the Electrometric Determination of Hydrogen-ion Concentration and for Gas Analysis 48
Historical Notes 65
Literature List 69

III. *Variability of Eggs and Sperm of Sea-urchins.* By A. J. Goldfarb 71-87
Technique 73
Concentration of the Germ-cells 74
Variation in Size and Shape of Eggs 77
Variations in Jelly Layer 78
Variation in Membrane Formation 79
Variation in Cleavage 80
Correlation of the Different Variations 84
Discussion 84
Conclusions 86
Bibliography 87

IV. *Analytical Search for Metals in Tortugas Marine Organisms.* By Alexander H. Phillips 89-93
Method 91

V. *On the Composition of Cassiopea xamachana and the Changes in it after Starvation.* By S. Hatai. 1 figure 95-109
Normal Growth of Different Parts of the Body 97
Analysis of Entire Body 99
Percentage of Water 99
Percentage of Nitrogen in Total Solids 99
Percentage of Ash in Solids 100
Analysis of Different Divisions of the Body 100
Percentage of Water 101
Percentage of Nitrogen in the Solids 101
Chemical Nature of the Gelatinous Substance of Cassiopea 102
Experiments on Starvation 103
Analysis of the Divisions of the Starved Cassiopea 108
Literature Cited 109

PAGE

VI. *The Habits and Reactions of a Comatulid, Tropiometra carinata.* By HUBERT LYMAN CLARK 111–119
Systematic Position 113
Habitat 114
Color and Size 114
Food 115
Locomotion 116
Reactions to Light 116
Reactions to Temperature 117
Reactions to Salinity 118
Reactions to Certain Chemicals 118
Summary 119

VII. *Studies on the Physiology of the Nervous System of Cassiopea xamachana.* By LEWIS R. CARY. 18 text-figures 121–170
Technique of the Experiments and Some Results of Operations that apply equally to all Experiments 125
Experimental 130
Influence of the Sense-Organs on the Loss in Weight during Starvation 149
Influence of Sense-Organs on the Rate of Central Metabolism as measured by CO_2 Production 155
Influence of Sense-Organs on the Change in Rate of Pulsation in Response to Changes in Temperature 159
Relation between Area of Tissue Enervated by a Single Sense-Organ and rate of Pulsation 161
General Discussion 164
The Relation of Muscular Activity to the Rate of Metabolic Processes in Cassiopea 164
Summary 166
Literature Cited 169

VIII. *The Chemistry of Light-Production in Luminous Organisms.* By E. NEWTON HARVEY. 1 text-figure 171–234
Studies on the Ostracod Crustacean *Cypridina hilgendorfii* 174
Studies on Fireflies 193
Studies on a Pennatulid, *Cavernularia haberi* 202
Studies on Luminous Bacteria 209
Studies on a Squid, *Watasenia scintillans* 222
Studies on a Fish, *Monocentris japonica* 223
Studies on Oxidative Chemiluminescent Reactions with Substances of Definite Composition 224
General Conclusions 233
Bibliography 234

IX. *A Physiological Study of Specific Gravity and of Luminescence in Noctiluca, with Special Reference to Anesthesia.* By ETHEL BROWNE HARVEY 235–253
Light Response in Noctiluca 237
Change of Osmotic Pressure 238
Salts of Sea-Water 241
Acids and Alkalies 242
Potassium Cyanide 243
Absence of Oxygen 244
Temperature 244
Centrifuging 245
Electricity 245
Response of Injured Cells 246
Location of Luminous Material 246
Anesthetics 247
Summary 251
Literature List 253

PAGE.
X. *Polychætous Annelids from Florida, Porto Rico, Bermuda, and the Bahamas.* By Aaron L. Treadwell. 3 plates 255–268
Family Syllidæ 257
Family Polynoidæ 258
Family Phyllodocidæ 262
Family Leodicidæ 263
Family Sabellidæ 266
Literature 268

XI. *The Microscopic Structure of Striped Muscle of Limulus.* By H. E. Jordan. 3 plates 273–290
Material and Methods 276
Skeletal Muscle 276
Cardiac Muscle 279
Literature 282
Discussion 283
Summary 287
Bibliography 289
Description of Illustrations 290

XII. *Hemopoiesis in the Mongoose Embryo, with Special Reference to the Activity of the Endothelium, including that of the Yolk-Sac.* By H. E. Jordan. 5 plates 291–312
Material and Methods 294
Hemopoiesis in Yolk-Sac 294
Cell-Clusters of the Aorta 298
Endothelial Hemogenesis in Intraembryonic Mesenchyma 301
Cell-Clusters of the Pericardium 304
Discussion 304
Addendum 305
Bibliography 311
Description of Illustrations 312

XIII. *Embryonic History of the Germ-Cells of the Loggerhead Turtle* (*Caretta caretta*). By H. E. Jordan. 6 plates 313–344
Material and Methods 316
Descriptive 319
Literature 327
Discussion and Conclusions 333
Summary of Results 340
Bibliography 342
Description of Illustrations 344

XIV. *Atresia of the Esophagus in the Embryo of the Loggerhead Turtle* (*Caretta caretta*)*: A Normal Developmental Condition.* By H. E. Jordan. 4 plates 345–360
Material 348
Descriptive 349
Discussion 354
Summary 358
Literature 360

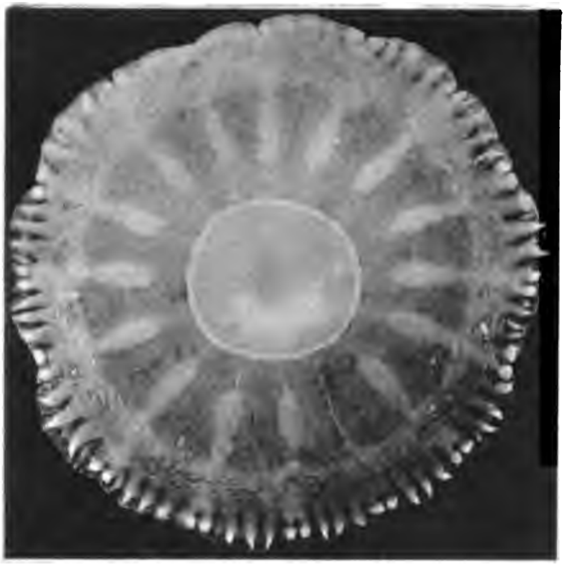
I.

NERVE-CONDUCTION IN CASSIOPEA XAMACHANA.

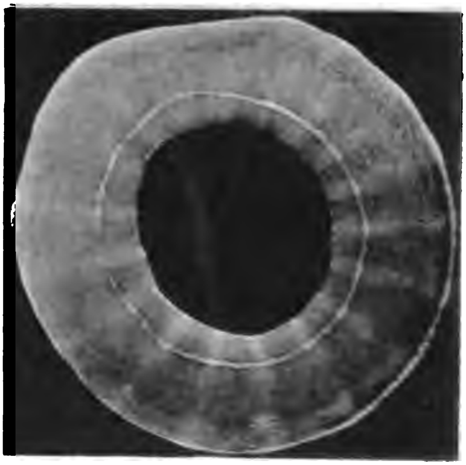
BY ALFRED GOLDSBOROUGH MAYER.

Fifteen figures.

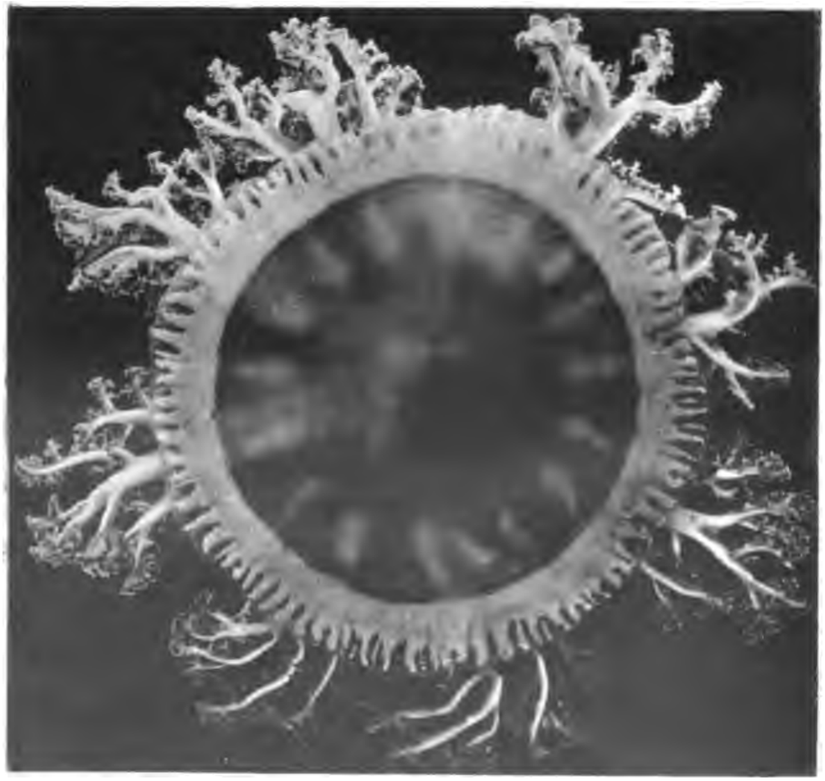
2



3



1



1. Aboral view of *Cassiopea xamachana* from the moat of Fort Jefferson, Tortugas, Florida.
2. Oral view of subumbrella of *Cassiopea xamachana* with stomach and mouth-arms removed.
3. Ring of subumbrella tissue made by cutting off the marginal sense-organs and removing center of disk.

NERVE-CONDUCTION IN CASSIOPEA XAMACHANA.

BY ALFRED GOLDSBOROUGH MAYER.

It is the author's privilege to acknowledge his indebtedness to kind friends for advice and aid: To Professor George A. Hulett, of Princeton University, for having had prepared in his laboratory the conductivity water used in this research; to Professor J. F. McClendon, of the University of Minnesota, for valued advice and aid, and to Professor L. R. Cary, of Princeton, for permission to make use of his recent determinations of the rate of nerve-conduction at different temperatures. By means of the generous interest of Professor E. G. Conklin and the authorities at Princeton I have enjoyed the excellent facilities afforded by the Biological Laboratory in Guyot Hall, wherein the kymograph records taken at Tortugas were studied and the results tabulated.

METHODS AND CORRECTIONS.

The object of this research was to obtain an accurate quantitative determination of the rate of nerve-conduction in natural and in diluted sea-water at constant temperature, and also to estimate the effects of various artificial sea-water solutions containing all or some of the sodium, magnesium, calcium, and potassium cations of sea-water. The effects of temperature upon nerve-conduction are also of great importance.

These studies were carried out in June and July 1916, upon *Cassiopea xamachana*, a rhizostomous scyphomedusa which is abundant in the salt-water moat surrounding Fort Jefferson at Tortugas, Florida, and is also common upon the bottoms of many of the shallow, semi-stagnant lagoons of the West Indian region. It is thus accustomed to a considerable range both in salinity and temperature, and being infested with commensal plant cells, it is in some measure independent of the oxygen-supply of the surrounding water, and even pulsates at a nearly normal rate in sea-water which has been deprived of air by boiling. The medusa thrives in confinement in glass aquaria and can be maintained alive in the laboratory for months while experiments are being performed upon it. Thus it is one of the most favorable of marine invertebrates upon which to conduct physiological studies.

An aboral view of *Cassiopea xamachana* is shown in figure 1, plate 1; figure 2 is an oral view of the subumbrella with the stomach and mouth-arms removed, and in figure 3 we see an annulus made by two circular cuts, one removing the marginal sense-organs and the other cutting out the central stomach, and in addition a circular scratch cutting through the nervous and muscular layer of the subumbrella, thus separating the broad annulus of tissue into two rings. This subumbrella tissue becomes paralyzed through the removal of the marginal sense-organs, but the outer of the two annuli may then be stimulated by an induction shock until a contraction-wave going in *one* direction is entrapped in it, as illustrated in figure 4, and as has been described by Mayer, 1906, 1908.* Such a neurogenic contraction wave must travel continuously through the circuit of tissue which has entrapped it, and may maintain itself for days with but little change of rate, provided the temperature, CO_2, salinity, and hydrogen-ion concentration of the sea-water remain constant. Thus we have a means of entrapping a single neurogenic stimulus which remains practically uniform in intensity and rate for any desired length of time.

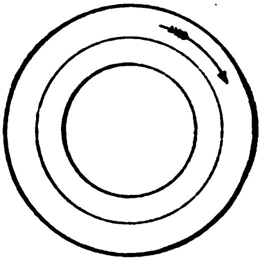


FIG. 4.—Diagram showing a single neurogenic pulsation-wave coursing in one direction through an annulus of subumbrella tissue.

Strong stimuli travel faster than weak ones, and thus if a wave stops it can not be started again at the same rate, for no two stimuli are received alike by the tissue. Such circuit waves may be stopped by counter-waves proceeding in the opposite direction against them, or blocked in the nerve net without apparent cause (fig. 5); or, if the tissue be exhausted, as in the absence of calcium, or by heat, cold, or CO_2, the wave may become irregular (fig. 13, lowest line) indicating that it is about to cease. In exhausted or weakened tissue, pulsus alternans may be displayed by the wave, the muscles becoming capable of responding fully only to every alternate (or even every second or third) return of the nerve stimulus (fig. 6, lowest line); but in normal, healthy tissues the wave is a full, regular sinusoid, the intervals being almost machine-like in their rhythmic sequence.

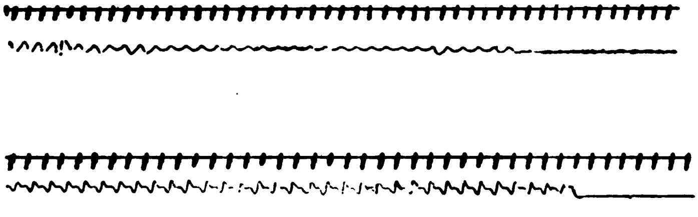


FIG. 5.—Two examples of sudden stopping of entrapped waves. Usually the wave becomes somewhat irregular before ceasing.

When a passive, paralyzed ring is activated by an induction shock, as in figure 7, muscular tonus is at once developed, and irregular and complex contraction waves proceed over it; but the larger waves travel faster than the smaller ones and thus overtake, and combine with them, in this manner forming a single component wave which appears upon the kymograph record as a smooth and regular sinusoidal curve.

---

*Carnegie Inst. Wash. Pub. No. 102, p. 116.

All chemicals used in this research were Merck's "Reagent" in quality.

In taking these kymograph records a Jaquet chronoscope beating seconds, or a make-and-break pendulum, was used with each individual line, thus making the accuracy of the record independent of any changes in rate of the kymograph drum. One ascertained the number of seconds required for the contraction wave to travel 100 times around the ring in pure sea-water; and then the number of times it traveled around the ring in the same number of seconds in diluted sea-water gave its percentage rate in the diluted sea-water.

The average temperature of the surface water of the ocean at Tortugas during the day time in June and July is about 29° C., and the experiments of Harvey (1911), Mayer (1914), and Cary (1916), show that the rate of nerve-conduction augments in practically a right-line ratio

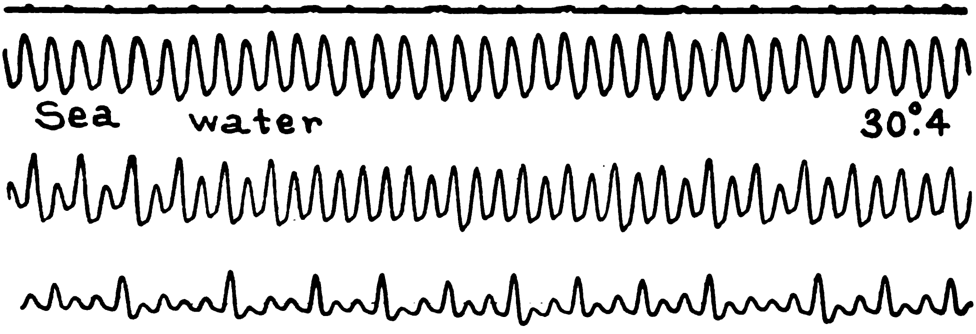


FIG. 6.—Pulsus alternans, indicating the weak, exhausted, or pathological character of the conducting tissue.

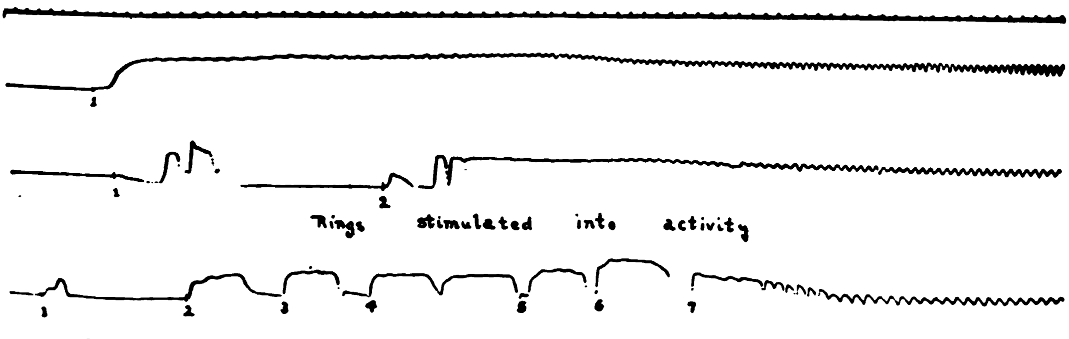


FIG. 7.—Three paralyzed rings being activated by induction shocks. A strong muscular tonus is at once developed, irregular waves proceed from the stimulated region, and finally a single regular sinusoidal wave going in *one* direction results from the interferences of the initial waves. The numbers represent successive attempts to stimulate each ring, the last one being successful.

from 17.5° to about 36° or 38° when it suddenly declines, the fall in rate indicating injurious effects, possibly due, as Harvey suggests, to the destruction of an accelerating enzyme. For several degrees, both above and below 29°, the rate changes about 4.5 per cent for each 1° C., and this correction was applied to all records. The records in diluted sea-water were usually taken at higher temperatures than those in the pure sea-water with which they were compared. The greatest average range was, however, not more than 1.79° C., but all records were reduced to the rate they would have exhibited had they been taken in a solution at the same temperature as that of the pure sea-water with which they were compared.

Another correction must be made on account of the osmotic interchange between the tissues of the ring and the diluted sea-water in which it is placed. Each experiment was made after the ring had been for one hour in 500 c.c. of fluid and a series of titrations with n/10 $AgNO_3$, using K_2CrO_4 as an indicator, showed the osmotic interchange had augmented the concentration of the diluted sea-water as follows:

TABLE 1.

Composition of the solution of 500 c.c. containing the pulsating ring.	Average percentage of concentration of the sea-water in which experiments were made.
Natural sea-water	100
95 vols. sea-water + 5 vols. distilled water	95.6
90 " " " + 10 " " "	90.56
80 " " " + 20 " " "	80.6
70 " " " + 30 " " "	70.8
60 " " " + 40 " " "	60.8
50 " " " + 50 " " "	50.8

The subumbrella tissue of *Cassiopea xamachana* is infested with commensal plant cells and thus when pulsating in diffuse daylight it gives out very little free CO_2. If placed in the dark, however, the sea-water surrounding the medusa soon becomes acid, due to the unreduced CO_2. All experiments were conducted during daylight hours in the diffuse light of the laboratory, and a series of tests showed that there was no appreciable change in the hydrogen-ion concentration of the pure or of the diluted sea-water after the pulsating ring had been in 500 c.c. of the solution for 1 hour. The laboratory was provided with a Leeds and Northrup potentiometer, which had been standardized by the U. S. Bureau of Standards; also with a Bovie potentiometer and two sets of standardized colorometric tubes made by Hynson, Westcott, and Dunning, one set being that of Rowntree, Levy, and Marriott, and filled with phenolsulphonephthalein, and the other set that of McClendon, wherein the tubes are filled with graded solutions of thymolsulphonephthalein. Both these sets of tubes were standardized by Professor McClendon, who was engaged upon a study of the sea-water as a physiological fluid. He found that the P_H of the surface sea-water at Tortugas in summer, at about 29° C., ranges from 8.1 to 8.2, the range being due to variations in its carbonates. As is well known from the studies of L. J. Henderson (1913), these carbonates act as a buffer substance, and by ionizing they tend to neutralize the effects of any slight accession of acid to the water. Thus McClendon found that sea-water of P_H 8.1 diluted with an equal volume of distilled water of P_H 6.04 gave a solution of P_H 8.09. Also 50 per cent sea-water plus 50 per cent distilled water of P_H 8 gives a solution which is fully as alkaline as, if not slightly more so than, pure sea-water.

A number of tests of the change in hydrogen-ion concentration after pulsating rings had been in 500 c.c. of various dilutions of sea-water showed that in daylight there was no considerable change in 1 hour; while in four others it declined similarly in pure sea-water after 12 hours, and in 3 other rings placed for 12 hours each in 500 c.c. of 60 per cent sea-water plus 40 per cent of 8 P_H distilled water the P_H had declined to 7.95. The average change at the end of an hour was certainly not greater than from 8.1 to 8.0 P_H.

The thermometer used in this research was compared with one which had been recently standardized by the U. S. Bureau of Standards; both thermometers read to 0.1° C.

Ordinary distilled water often retains an acid reaction even after air freed from CO_2 by passing through tubes containing granulated soda-lime has been bubbled through it for 72 hours. Accordingly, Professor George A. Hulett, of Princeton University, very kindly offered to have prepared, under his direction and by his well-known method,* 144 liters of distilled water, which were sealed in 144 clean, steamed flasks of pyrex glass.† The hydrogen-ion concentration of the contents of each flask was tested separately and found to range from 0.80 to 1.0×10^{-6}, the average being 0.9×10^{-6}, or about 6.045 P_H.

This water was taken to Tortugas and used in all experiments herein mentioned. It retained a constant hydrogen-ion concentration and apparently no injurious elements were derived from the pyrex glass. In the series of experiments wherein the sea-water was diluted with distilled water of about 6 P_H, each pyrex glass flask was opened by breaking the narrow neck of the flask and the water was used as soon as possible, the opening in the neck of the flask being sealed in the intervals by a plug of soft paraffine. The distilled water of 6 P_H used in diluting the sea-water was not aerated and was thus deficient in oxygen. This, however, makes but little difference, for the commensal plant cells in the tissues of *Cassiopea* are so active in diffuse daylight that the medusa pulsates at a nearly normal rate even if placed in sea-water which had been boiled for an hour to expel its air, then restored to its former volume by air-free distilled water and cooled to normal temperature. Thus in daylight the medusa appears to be able, due to its plant cells, to supply nearly if not quite enough oxygen for its normal metabolism. The presence of free CO_2 in the surrounding sea-water is, however, very toxic and soon stops pulsation, and in darkness unreduced CO_2 accumulates, and thus the rate of movement is considerably slower than in daylight.

---

*1896; Zeitschift phys. Chemie, Bd. 21, p. 287.

†This water was prepared and tested by Mr. J. H. Yoe in Professor Hulett's laboratory.

In another series of experiments, it was desired to dilute sea-water with distilled water of the same hydrogen-ion concentration as that of the sea-water itself. Accordingly, 50 flasks of Professor Hulett's distilled water were broken and the contents poured into a green-glass carboy which had previously contained Merck's distilled water. Air which had been freed from CO_2 by drawing it through glass tubes containing granulated soda-lime was then bubbled vigorously through this distilled water for 78 hours, after which the water had a P_H of 8. It was protected from the carbon dioxide of the atmosphere by permitting only air which had passed through soda-lime tubes to enter the carboy, and thus it retained an unchanged alkalinity remaining 8 P_H from July 4 to 11 inclusive, during which time 139 dilution experiments were conducted with it; after this its alkalinity began to decline while 26 additional experiments were made. The average for the entire series was 7.93 P_H or a hydrogen-ion concentration of 1.17×10^{-8}.

The fixity of the alkalinity at P_H 8 for so many days was probably due to OH ions derived from the alkaline green glass of the carboy, which may have been counterbalanced by a slight leakage of CO_2 through the rubber stopper of the carboy. Then, when the volume of the water became reduced, the surface over which solution of glass took place was relatively reduced in comparison with the surface capable of absorbing atmospheric CO_2, and thus the water finally tended toward acidity. Previous to its being used to hold the distilled water the carboy was cleaned by washing it with solutions of HCl, distilled water, KOH, absolute alcohol, and finally distilled water, taking precautions to prevent dust from entering.

The rings cut from the subumbrella of *Cassiopea* were attached to the kymograph lever by threads of catgut (fig. 15); while the rings themselves were each placed in 500 c.c. of solution contained in clean glass jars, covered between times of record-taking to prevent evaporation and to exclude dust.

Each ring was first placed in 500 c.c. of sea-water and its rate and temperature ascertained. It was then transferred to another glass jar containing 500 c.c. of a freshly made solution composed of 475 c.c. of sea-water and 25 c.c. of distilled water. At the end of an hour its rate was again ascertained on the kymograph and it was transferred to another glass vessel which contained 90 per cent sea-water plus 10 per cent distilled water, and thus it was tested in more and more dilute solutions down to 50 per cent sea-water plus 50 per cent distilled water, after which it was replaced in natural sea-water. Rings which have been in 50 per cent sea-water recover more than 80 per cent of their normal rate within an hour after being replaced in pure sea-water, and, if replaced from 70 per cent sea-water, recovery is practically complete at the end of an hour. Even 50 per cent dilution produces, however, little or no permanent injury.

EXPERIMENTS.

Studies of recent years have shown the important influence of hydroxyl and hydrogen ions upon the rate of nerve-conduction. The free hydroxyl ion is a stimulant through a considerable range of concentration, but the free hydrogen ion is a depressant, excepting that in very weak concentration it acts as a moderate stimulant. Thus, if sea-water be diluted with acid distilled water of about P_H 5.5 (H-ion concentration 0.316×10^{-5}) the rate of nerve-conduction is augmented in slight dilutions, but it declines more and more rapidly in dilutions of 80 per cent sea-water plus 20 per cent distilled water and over. The muscular activity, as measured by the amplitude of the pulsation wave, usually declines steadily in sea-water diluted with acid distilled water of P_H 6, while when diluted with alkaline water of P_H 8 it maintains itself or even augments.

Table 2 shows the effect upon the muscular activity as measured by the amplitude of the pulsation wave in *Cassiopea* when taken from natural sea-water and gradually run into diluted sea-water composed of 50 volumes sea-water plus 50 volumes distilled water (50 per cent sea-water), at 30° C.

TABLE 2.

No. of experiments.	Hydrogen-ion concentration of the distilled water used in diluting the sea-water to 50 per cent.	Percentage of cases in which muscular activity was augmented by dilution of the sea-water.	Percentage in which muscular activity was decreased by the dilution of the sea-water.	Percentage unaffected by the dilution.
22	Acid distilled water of P_H about 6 at 30° C.	23	59	18
28	Alkaline distilled water of P_H 7.93 at 30° C...	57	25	18

Thus when sea-water is diluted with alkaline distilled water, more than half the rings show augmented muscular activity, whereas if the distilled water be slightly acid the muscles of more than half are depressed, and this depression is much more marked the greater the acidity of the distilled water used in diluting, so that with distilled water of about 5.5 P_H the amplitude of muscular movement is hardly perceptible in 50 per cent sea-water. As the average temperature of the sea-water was about 30° C., the neutral point was about 6.86 P_H and thus it appears that even very slightly acid distilled water is usually a muscular depressant, whereas alkaline distilled water is a correspondingly efficient stimulant; this is the more remarkable when we consider that the hydrogen-ion concentration of the sea-water is very slightly augmented by dilution even with its own volume of distilled water of 6 P_H. Thus McClendon found that such a dilution of sea-water of 8.1 P_H changed the P_H of the solution to 8.09; in other words, the relative concentration of the hydrogen ions was increased only from 1 to 1.02, such is the efficiency of the buffer carbonates in preserving the normal alkalinity of the sea-water.

It will be recalled that Osterhout (1914)* finds that a slight concentration of HCl is at first stimulating to plants, but later (after the plasma membranes have been penetrated by the acid) it becomes depressant. It would seem that in *Cassiopea* a very slight relative increase of the H ion can not penetrate the cells, but acts only on the cell surfaces or membranes and in so doing becomes a stimulant; but in stronger concentration the cell membranes are penetrated and the H ion then exerts its well-known depressant effect.

The muscles are much more sensitive to changes of concentration in $H^{\cdot}$ or OH' ions than are the nerves.

The stimulating effect of a very slight increase of acidity or of alkalinity upon the rate of nerve-conduction is well shown if we place the pulsating ring in natural sea-water and then add about 5 per cent of acid distilled water of P_H about 5.5, for the rate suddenly augments about 5 per cent. Similarly, if we take the pulsating ring from normal sea-water and place it in sea-water which has been condensed by evaporation at normal temperature in the sun to about 90 per cent of its original volume, the rate augments on account of the high alkalinity of the evaporated sea-water.

Professor McClendon made up a series of acid and alkaline sea-waters. He added 23 c.c. of n/10 $NaHCO_3$ to each 1,000 c.c. of natural sea-water and after bubbling air through the solution for 12 hours the P_H was 8.26, and a film of calcium carbonate was precipitated upon the sides of the glass flask containing the solution. Similarly, he made acid sea-waters of P_H 7 by adding to each liter 24 c.c. of n/10 HCl and then aerating for 12 hours; and a sea-water of P_H 5.6 was obtained in the same manner by aerating for only half an hour.

The rates of nerve-conduction are nearly normal in all these solutions; but although the differences in rate are slight, they are such as we expected, the alkaline sea-water and the weakly acid sea-water are both slightly stimulating, while the more acid sea-water is depressant, as shown in table 3, the temperature of the solutions being 29° C. and the neutral point 6.88 P_H.

---

*Journ. Biol. Chemistry, vol. 19, p. 518.

TABLE 3.

Character of the sea-water.	Average rate of nerve-conduction.	No. of observations.
Alkaline sea-water of P_H 8.26......	102	6
Natural sea-water of P_H 8.15......	100	....
Nearly neutral sea-water of P_H 7...	99.9	5
Slightly acid sea-water of P_H 6.5...	102	6
Acid sea-water of P_H 5.6..........	99.4	6

The effects are not so marked as those which are observed when the pulsating rings are taken from natural sea-water and placed in sea-water diluted with slightly acid distilled water, or in sea-water made alkaline by being concentrated by evaporation at ordinary temperatures exposed to the air; but they accord with expectation in so far as they go. Possibly the OH′ anion is stimulating even after it penetrates the cell membranes, while the hydrogen cation is depressant under these conditions.

The effects upon the rate of nerve-conduction in *Cassiopea* which resulted from diluting Tortugas sea-water with alkaline distilled water of P_H about 8 and with slightly acid distilled water of P_H about 6 are given in table 4, which shows that acid distilled water is slightly stimulating in comparison with alkaline distilled water in weak dilution down to about 75 per cent sea-water plus 25 per cent distilled water, and then becomes relatively depressant in stronger dilutions. There are many examples of this in the author's past experiments published in 1914, 1915, and 1916, wherein the distilled water used for dilution was usually acid. An interesting parallel

TABLE 4. (Illustrated by figure 8.)

Composition of the solution.	Rate of nerve-conduction when sea-water is diluted with alkaline distilled water of P_H 7.93 at 29° C.	Rate of nerve-conduction when sea-water is diluted with slightly acid distilled water of P_H 6.04 at 29° C.
Natural sea-water of P_H 8.1 to 8.2........	100	100
95 c.c. sea-water + 5 c.c. distilled water..	96.23	96.43
90 " " " + 10 " " "	91.44	94.38
80 " " " + 20 " " "	79.51	82.68
70 " " " + 30 " " "	73.91	71.95
60 " " " + 40 " " "	65.72	60.41
50 " " " + 50 " " "	54.16	50.83

appears to be that of acidosis in blood, wherein pathological effects appear when the amount of acid is so slight that the P_H is hardly affected, the buffer value of the carbonates and phosphates, however, being reduced.

It was due, indeed, to the acidity of the distilled water, and to my not realizing the marked effect of slight changes in temperature, that I was misled into entertaining the false idea that the curve of decline in rate in diluted sea-water resembles that of Freundlich's curve of adsorption, and that therefore adsorption may play a fundamental rôle in nerve-conduction. These recent experiments, wherein the sea-water was diluted with distilled water of P_H about 8, appear to show that adsorption has nothing to do with the matter.

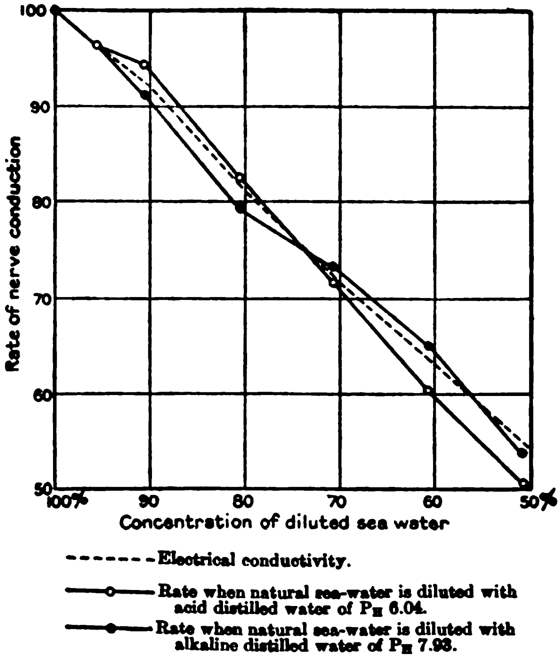


FIG. 8.—Showing decline in rate of nerve-conduction in *Cassiopea* in diluted sea-water, and corresponding decline in the electrical conductivity of sea-water. See tables 4 and 5.

Indeed, Ralph S. Lillie (1916)* has already called attention to the close resemblance between my curve for decline in rate of nerve-conduction and the corresponding decline in the electrical conductivity of the diluted sea-water, and in this latest and best-established curve of 1916, using distilled water of about P_H 8 to dilute the sea-water, the accordance with Lillie's expectation is almost perfect, as appears from table 5 and figure 8. It should be said, however, that the degree of ionization of the sodium, calcium, and potassium of the sea-water follows nearly the same law. Thus the rate of nerve-conduction, as we shall show, appears to be directly proportional to the *concentration* of the surrounding cations of sodium, potassium, and calcium, magnesium taking an almost negative part in the control of rate of nerve-conduction in diluted sea-water.

---

*American Journal of Physiology, vol. 41, pp. 126–136.

Table 5 (illustrated by fig. 8) shows the rates of nerve-conduction in *Cassiopea* in Tortugas sea-water diluted with aerated, alkaline distilled water having a hydrogen-ion concentration of 1.17×10^{-8}, or 7.93 P_H.

TABLE 5.

Composition of the solution.	Average rate of nerve-conduction.	Electrical conductivity of Tortugas sea-water diluted with distilled water of P_H 7.8 at 30° C. determined by Kohlrausch's method.
Natural sea-water of P_H 8.1 to 8.2	100	100
95 c.c. of sea-water + 5 c.c. of distilled water	96.23	......
90 " " " + 10 " " " "	91.44	92.16
80 " " " + 20 " " " "	79.51	81.38
70 " " " + 30 " " " "	73.91	71.53
60 " " " + 40 " " " "	65.72	64.26
50 " " " + 50 " " " "	54.16	54.08

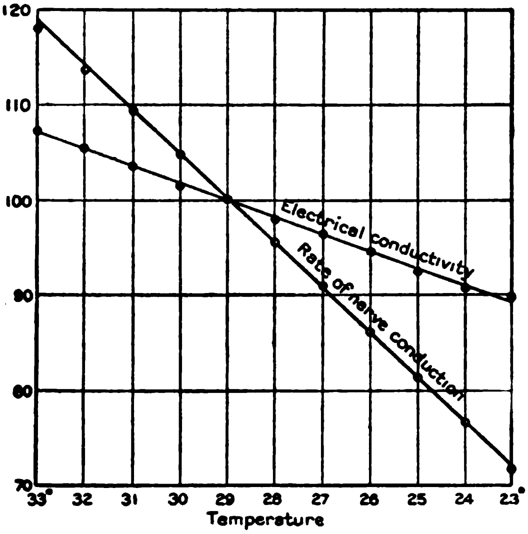


FIG. 9.—Showing that the temperature coefficient for the rate of nerve-conduction in *Cassiopea* is 2.5 times as great as that of the electrical conductivity of the sea-water.

The close resemblance between the decline in rate of nerve-conduction and the corresponding decline in electrical conductivity of the surrounding sea-water suggests but does not prove that the two phenomena are directly dependent one upon the other; but as we shall show later, this seems doubtful.

Lillie advocates an extension of the theory of Faraday and de la Rive that the transmission of the excitation state from the immediate site of activity to the adjoining resting areas is dependent upon an electrical local action of the same essential nature as that which is responsible for the etching or corrosion of non-homogeneous metallic surfaces, such as iron in contact with an electrolyte solution. Lillie calls this theory the "local action theory of conduction," and if, indeed, nerve-conduction be such a process, its rate must be proportional to the electrical conductivity of the conducting medium and the surrounding fluid (sea-water).

Lillie's hypothesis is, moreover, indirectly supported by the recent work of Adrian (1916),* who shows that after stimulation the recovery of conductivity is apparently complete at the same instant when the

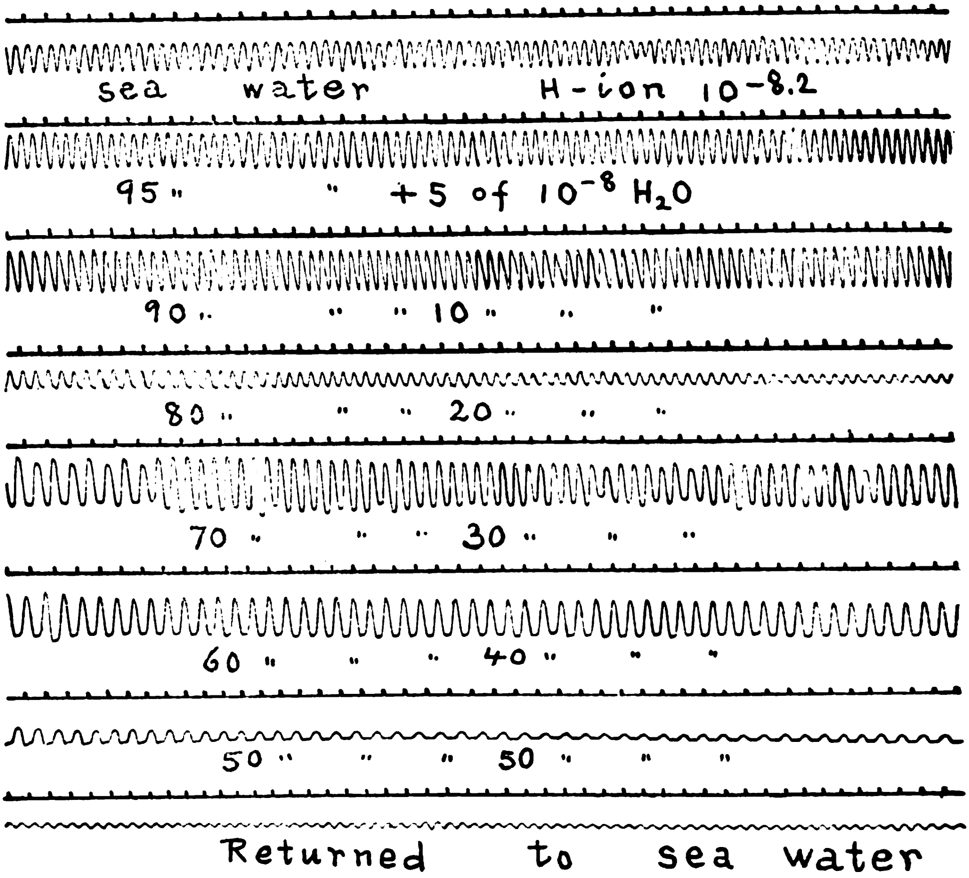


FIG. 10.—A ring of *Cassiopea* in diluted sea-water, showing decline in rate of nerve-conduction as dilution proceeds, followed by recovery of rate but not of amplitude upon being replaced in natural sea-water.

---

*Journal of Physiology, Cambridge, vol. 50, p. 345.

recovery of excitation is also completed and thus the two mechanisms may be identical and conduction may be merely the spread of a local exciting process.

Contrary to Lillie's hypothesis, we have direct evidence that the rate of nerve-conduction may be independent of the electrical conductivity of the electrolytic solution surrounding the nerves, for I have found (1915)* that if sea-water be diluted with 0.415 molecular $MgCl_2$ the rate of nerve-conduction is only slightly more depressed than if the sea-water be diluted with distilled water, or with dextrose; yet the $MgCl_2$ maintains a nearly normal electrical conductivity, while with distilled water or dextrose it declines in nearly the same ratio as the dilution. Nor do the experiments I have made with solutions containing some but not all the cations of sea-water support Lillie's view. Thus if the rate of nerve-conduction in 0.647 molecular NaCl be 55, it becomes 100 in 85.3 c.c. of 0.6 molecular NaCl+14.69 c.c. of 0.39 molecular $MgCl_2$. Here the electrical conductivity of the solution is somewhat reduced,

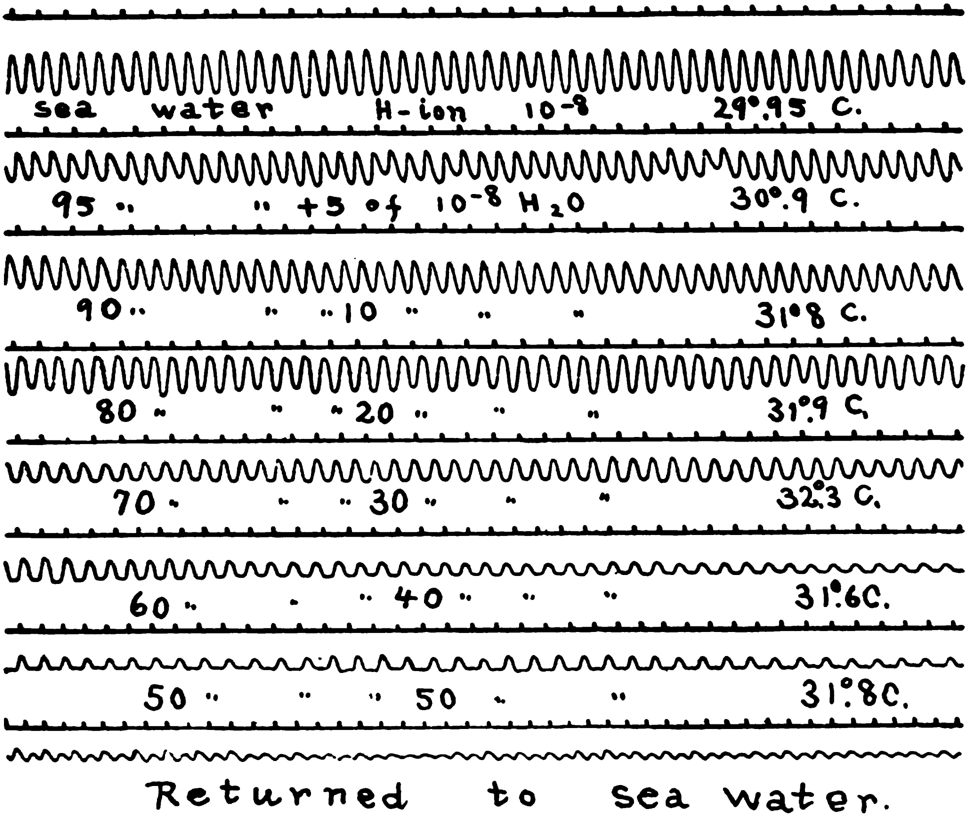


FIG. 11.—Showing changes in rate of nerve-conduction in a ring of *Cassiopea* in successive dilutions of sea-water, and the recovery of rate on being returned to natural sea-water from 50 per cent.

---

*Proceedings National Acad. Sciences, vol. 1, p. 270.

while the rate of nerve-conduction is much augmented. This is, of course, a striking instance of Loeb's law of the antagonism between a univalent and a bivalent cation, even though the bivalent cation in this case is magnesium, well known to be a depressant, especially for muscular activity in *Cassiopea.*

The temperature coefficient of nerve-conduction is 2.5 times as great as that of the electrical conductivity of the sea-water, as appears in table 6.

The rate of nerve-conduction in *Cassiopea* in heated sea-water was first determined by Harvey (1911) who found it to accelerate in a right-line ratio up to 35° to 38° C., at which point there was an abrupt decline in rate. These results were later confirmed by Mayer (1914), and Cary (1916). The average of the best experiments is shown in table 6.

Table 6. (Illustrated by figure 9.)

Temperature of the sea-water, °C.	Rate of nerve-conduction in *Cassiopea.* (Mean of determinations of Harvey, Mayer, and Cary.)	Electrical conductivity of Tortugas sea-water, determined by Kohlrausch's method.
23	71.3	88.9
24	76.3	90.7
25	81.17	92.6
26	85.8	94.4
27	90.74	96.2
28	95.47	98
29	100	100
30	104.47	101.6
31	109.2	103.5
32	113.4	105.3
33	117.8	107.1

The high temperature coefficient of the rate of nerve-conduction suggests that we may be dealing with a chemical reaction involving a compound composed of sodium, calcium, and some proteid element; the degree of ionization of which is considerably affected by temperature in the manner suggested by W. B. Hardy (1900), Quincke (1902), and Bayliss (1915).*

Possibly, also, the negative electrical potential associated with the wave of nerve-conduction may increase the surface tension of the alkaline colloidal particles, thus reducing their size, rendering them more soluble, and thereby increasing the concentration of the reacting ions.

In this connection, A. Mayer, A. Schaeffer, and E. Terroine (1907)† state that in a large number of alkaline organic colloids, the addition of a further negative charge, in the form of OH′ ions, caused a decrease in the size of the particles. Moreover, Hulett (1901)‡ has shown that the solubility of particles of barium sulphate is about inversely proportional to the size of the particles; and as colloidal particles are more or less soluble, one would expect their solubility to increase as the average diameter of the particles decreased.

---

*Principles of General Physiology, p. 77.

†Compt. Rendus Acad. Sci., Paris, tome 145, p. 919.

‡Zeitschrift physik. Chemie, Bd. 37, p. 406.

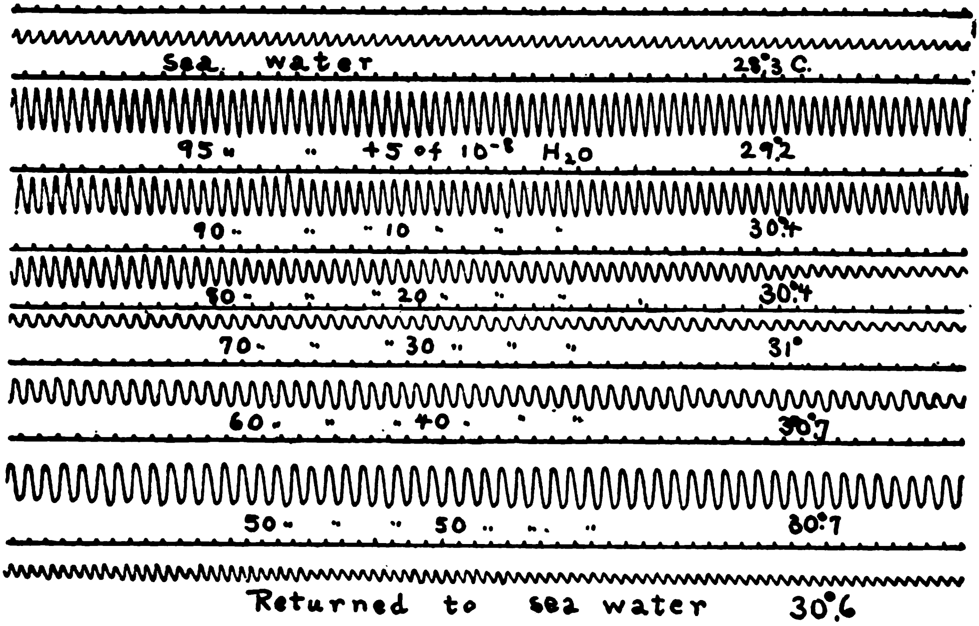


FIG. 12.—A ring of *Cassiopea* in diluted sea-water, showing decline in rate of nerve-conduction as dilution proceeds; also recovery of rate when the ring is replaced in natural sea-water.

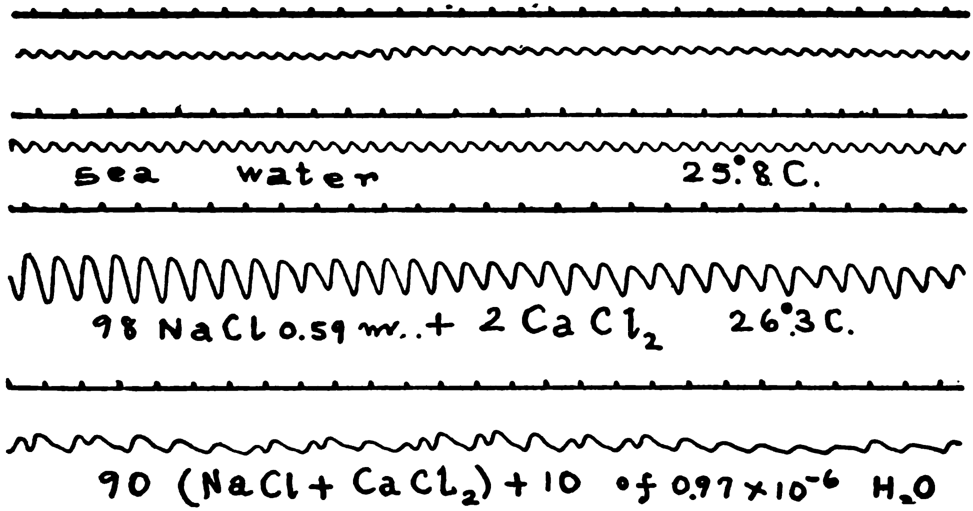


FIG. 13.—Showing change in rate of nerve-conduction in subumbrella ring of *Cassiopea* in $NaCl+CaCl_2$. In the lowest line the movement of the ring has become very irregular, indicating that it is about to stop.

SUMMARY.

Nerve-conduction is due to a chemical reaction involving the cations of sodium, calcium, and potassium. Magnesium is non-essential.

The probably high temperature coefficient of ionization of this ion proteid may account in some measure for the high temperature coefficient of the rate of nerve-conduction, which I find is 2.5 times as great as that of the electrical conductivity of the sea-water surrounding the nerve.

Our observations do not support the "local action" theory of R. S. Lillie (1916); for this maintains that the rate of nerve-conduction must

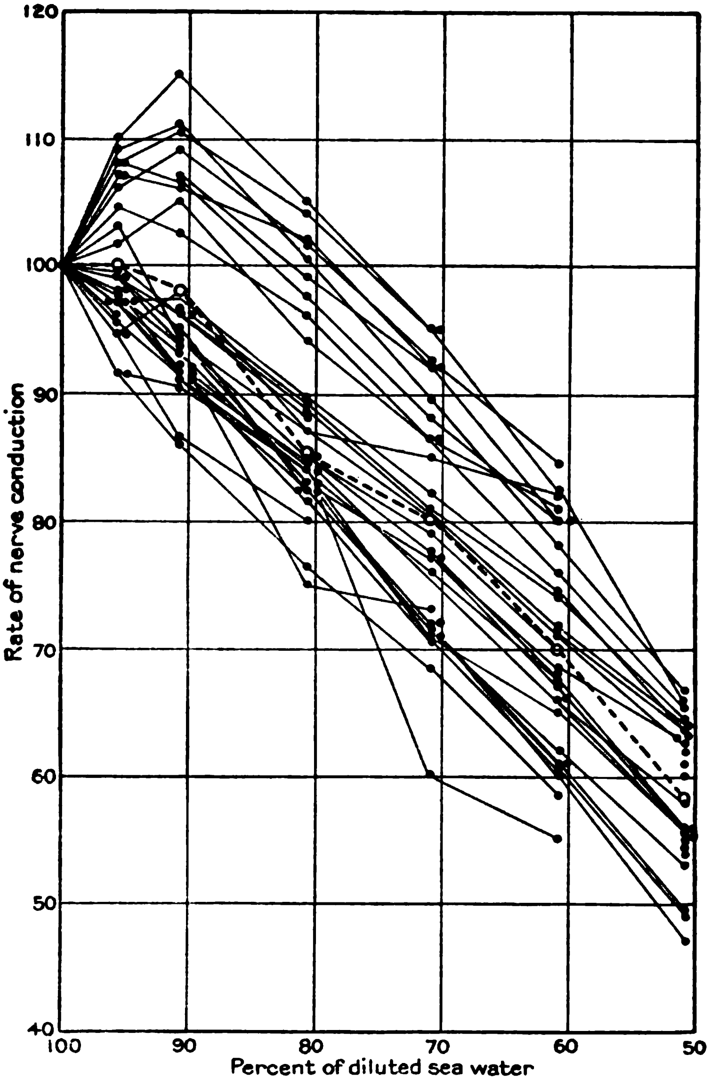


Fig. 14.—Illustrating table 7. Results of each individual experiment upon rate of nerve-conduction in *Cassiopea* in sea-water diluted with alkaline distilled water of hydrogen-ion concentration 1.17×10^{-8}. The heavy dotted line shows the observed average curve.

be a function of the electrical conductivity of the conducting tissue and of the electrolytic solution surrounding the nerve. It is found, however, that the rate of nerve-conduction is practically identical, whether we dilute sea-water with 0.415 molecular $MgCl_2$ or with distilled water—in other words, whether we maintain a practically constant electrical conductivity or reduce it in nearly the same ratio as the dilution.

Table 7, illustrated by figure 14, shows rates of nerve-conduction in the subumbrella tissue of *Cassiopea xamachana* in Tortugas sea-water of P_H 8.1 to 8.2 diluted with aerated distilled water of P_H about 8.0 at 29° C. The rate in pure sea-water at the same temperature as the diluted sea-water is assumed to be 100. The experiments were conducted at Tortugas, Florida, on July 4 to 11 and July 19 to 25, 1916.

TABLE 7.

[s.w. = sea-water. d.w. = distilled water.]

	Natural sea-water.	95 c.c. of s.w. + 5 c.c. of d.w.	90 c.c. of s.w. + 10 c.c. of d.w.	80 c.c. of s.w. + 20 c.c. of d.w.	70 c.c. of s.w. + 30 c.c. of d.w.	60 c.c. of s.w. + 40 c.c. of d.w.	50 c.c. of s.w. + 50 c.c. of d.w.
Average concentration of sea-water at time record was taken.....	100	95.6	90.56	80.6	70.8	60.8	50.8
Number of observations.	38	28	28	28	28	29	24
Observed average rate...	100	100.1	97.87	85.38	80.05	70.11	58.37
Probable error of observed average rate...	0	0.67	1.00	1.18	1.16	1.01	0.79
Temperature difference (°C.) between solution and pure sea-water in which rate was 100...	0°	0.86°	1.46°	1.53°	1.79°	1.51°	1.64°
Rate corrected for temperature by reducing observed rate 4.5 per cent for each 1° C. rise in temperature.......	100	96.23	91.44	79.51	73.91	65.72	54.16

Observed rates.

95 c.c. of s.w. + 5 c.c. of d.w.		90 c.c. of s.w. + 10 c.c. of d.w.		80 c.c. of s.w. + 20 c.c. of d.w.		70 c.c. of s.w. + 30 c.c. of d.w.		60 c.c. of s.w. + 40 c.c. of d.w.		50 c.c. of s.w. + 50 c.c. of d.w.	
91.5	99	86	96	75	87	60	80	55	70	47	62
91.5	99	86.5	96	76.5	88	68.5	80.5	58.5	71	49	62.5
94.5	99.5	90.5	96.5	80	88.5	70.5	81	60	71.5	49.5	63
94.5	100	91	97.5	81.5	89	71	82	60.2	71.7	53	63.2
95.5	101.5	91	98	82.5	89.7	71	85	61	74	54	63.5
96	103	91.5	102.5	82.5	94	71.5	86.5	61	74.5	54.5	64
97	104.5	92	105	82.5	96	72	86.5	62	76	55	64.5
97	106	92	106	83	97.5	72	88	65	78	55.5	65.5
97	107	93	106.5	83	99	73	89.5	66	80	55.5	66
97	107	93.5	107	84	100.4	76	92	66	80	56	66.7
97.5	108	93.5	109	84	101.5	77	92	67	81	56	....
98	108	94	110.7	84.5	102	77	92.5	67.5	82	58	....
98	109	94.5	111	85	104	77.5	95	68	82.5	60	....
98	110	95	115	85	105	79	95	68.5	84.5	61	....
Average 100.1		Average 97.87		Average 85.38		Average 80.05		Average 70.11		Average 58.37	

Table 8 shows the rates of nerve-conduction in subumbrella tissue of *Cassiopea xamachana* in Tortugas sea-water of P_H 8.1 to 8.2 diluted with non-aerated distilled water of P_H about 6 (H-ion concentration 0.9×10^{-6}) at 29° C. The rate in undiluted sea-water is taken to be 100. The experiments were conducted at Tortugas, Florida, in June and July 1916.

TABLE 8.

[s.w. = sea-water. d.w. = distilled water.]

	Natural sea-water.	95 c.c. of s.w. + 5 c.c. of d.w.	90 c.c. of s.w. + 10 c.c. of d.w.	80 c.c. of s.w. + 20 c.c. of d.w.	70 c.c. of s.w. + 30 c.c. of d.w.	60 c.c. of s.w. + 40 c.c. of d.w.	50 c.c. of s.w. + 50 c.c. of d.w.
Average concentration of sea-water at time record was taken.....	100	95.6	90.56	80.6	70.8	60.8	50.8
Number of observations.	51	20	27	25	28	26	22
Observed average rate...	100	99.1	97.91	87.96	77.28	64.33	53.79
Probable error of observed average rate...	0	0.74	0.88	1.16	1.13	0.59	0.90
Temperature difference (°C.) between solution and pure sea-water in which rate was 100...	0°	0.6°	0.8°	1.33°	1.54°	1.37°	1.23°
Rate corrected for temperature by reducing observed rate 4.5 per cent for each 1° C. rise in temperature.......	100	96.43	94.38	82.68	71.95	60.41	50.83

Observed rates.

95 c.c. of s.w. + 5 c.c. of d.w.		90 c.c. of s.w. + 10 c.c. of d.w.		80 c.c. of s.w. + 20 c.c. of d.w.		70 c.c. of s.w. + 30 c.c. of d.w.		60 c.c. of s.w. + 40 c.c. of d.w.		50 c.c. of s.w. + 50 c.c. of d.w.	
94	98	85	98	76.5	88	62.5	74.5	54	66.5	41	54
96	98	86	98.5	77	88	67	75	55.4	66.5	43.5	55
95.5	98	88	99	77.5	88	69.5	76.5	59.5	66.5	46.5	55.5
96	98	91	99.5	79.5	89	70	76.5	60	67	47	56.5
96.5	99	92	100	82	90.2	71	77.5	60	68	50	57.5
97	103	92	100.5	82	90.5	71	79	60.5	68	50	58.5
97	103	94	101	83	91	72.5	79.5	60.5	68.5	52	....
97.5	106	94	102	84.2	96	73	80	61	69.5	52.5	....
97.5	107	94.5	104	85	106	73	80.5	62	69.5	53	....
97.5	108.5	96	104	85	107	73.5	80.5	64	....	53	61
....	.....	96	105	85.5	109	74	82	64.5	70	53.5	62
....	.....	96	107.5	85.5	.....	74	83	64.5	72	53.5	63
....	.....	97	111	86.5	.....	74.5	91.5	64.7	....	....	65
....	.....	....	112	87	.....	....	96	65	....	....	....
....	99.1	....	.....	....	.....	....	107	66	....	....	....
Average 99.1		Average 97.91		Average 87.96		Average 77.28		Average 64.33		Average 53.79	

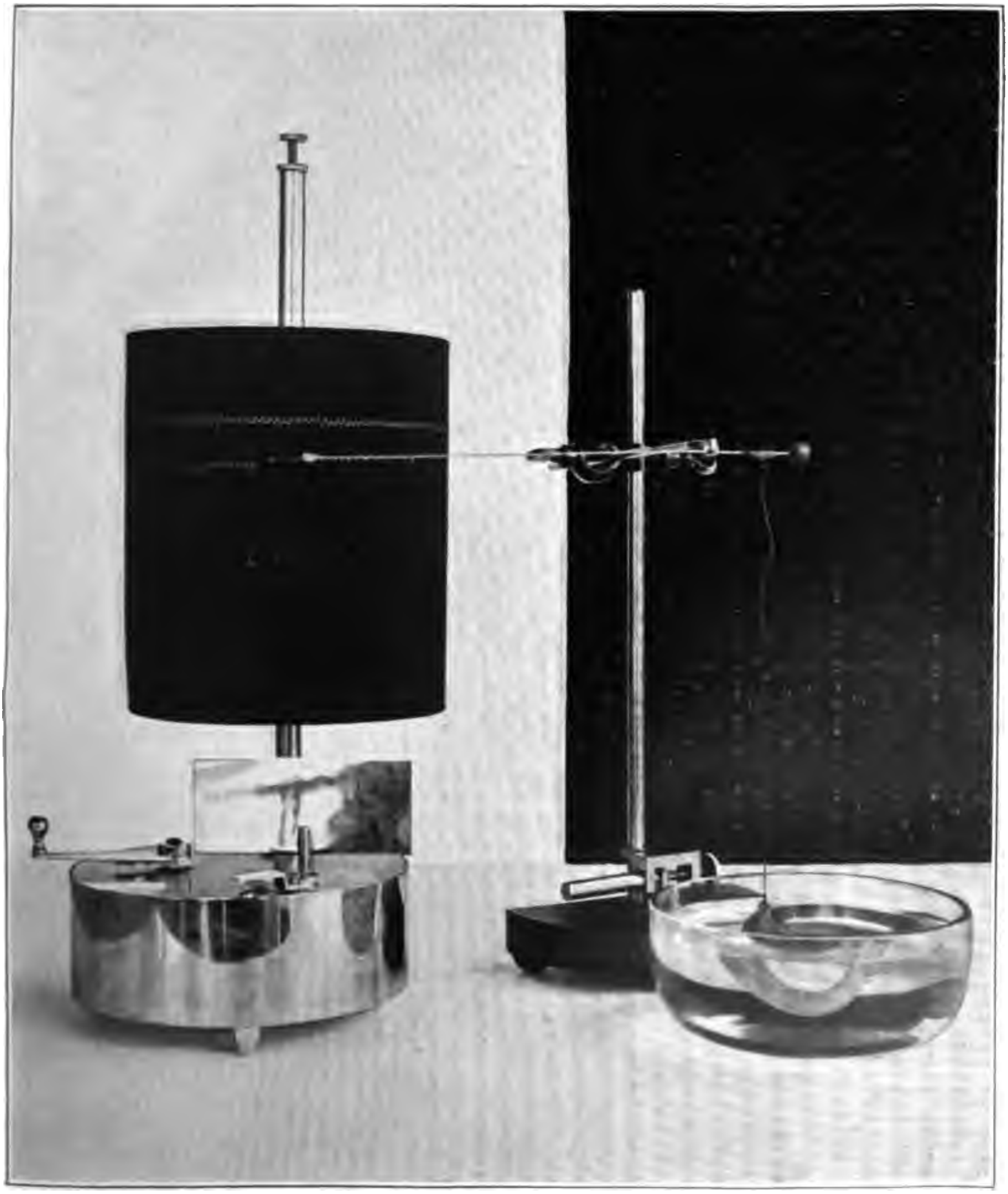


Fig. 15.—Taking a Kymograph Record from a Pulsating Ring. The chronoscope is not shown.

II.

THE HYDROGEN-ION CONCENTRATION, CO_2 TENSION, AND CO_2 CONTENT OF SEA-WATER.

BY J. F. McCLENDON, C. C. GAULT, AND S. MULHOLLAND.

Twenty-four figures.

CONTENTS.

PAGE.
Definitions and abbreviations 23
Results applied to oceanography and measurement of respiration-rate of marine organisms 27
Calibration of an improved indicator method for use on shipboard and in poorly equipped laboratories 42
New apparatus for the electrometric determination of hydrogen-ion concentration and for gas analysis 48
Historical notes 65
Literature list 69

THE HYDROGEN-ION CONCENTRATION, CO_2 TENSION, AND CO_2 CONTENT OF SEA-WATER.*

BY J. F. McCLENDON, C. C. GAULT, AND S. MULHOLLAND.

DEFINITIONS AND ABBREVIATIONS.

Since the volume of water changes with the temperature, the most accurate method of expressing the results of water analysis is to take the kilogram of water as the unit. The older method was to express results in grams per kilogram. In volumetric analysis it is more convenient to express results in grams per liter, and this method is equally accurate provided the water is brought to a standard temperature, preferably 20°, for analysis, or the temperature is recorded in each case. Since the ratios of the weights of chemical elements or radicals that will enter into chemical composition with one another have been established with an accuracy more than adequate for oceanographic work, it is still more convenient to express results in gram-equivalents per liter.

A normal solution (abbreviated to n) is a solution containing in 1 liter the quantity of substance that will combine with 23 grams of sodium or 35.46 grams of chlorine. A mol-liter solution (abbreviated to m) is the molecular weight in grams (mol) of the substance contained in a liter of the solution, and may or may not be a normal solution, and where confusion is likely to arise, m and not n should be used. For example: if enough H_3BO_3 is added to a normal solution of NaOH to make a 2 m solution of the former, the result is a 0.5 m solution of $Na_2B_4O_7$. In the ordinary use of m we mean more correctly the *formal* concentration—*i. e.*, the molecular weight is taken from the ordinary chemical formula, regardless of the fact that several molecular species containing the substance in question may occur in the solution.

Where a particular molecular or ionic species is intended, it should be designated. Thus a normal solution of hydrogen ions contains 1.008 grams (or for practical purposes about 1 gram) of hydrogen ions per liter, regardless of the amount of hydrogen in other chemical states. It is not always possible to reduce such a quantity to a standard temperature, since some hydrogen ions may disappear or be formed during the change in temperature. This particular case is simplified by the fact that the normal concentration of H ions per liter is not changed very much by the ordinary changes in temperature, as will be explained later. It is more convenient to abbreviate the names of positive and negative ions thus: H˙ = hydrogen ion, Cl′ = chlorine ion.

---

*This investigation was aided by a grant from the research fund of the graduate school of the University of Minnesota. We are indebted to Dr. H. A. Lubs and Professor L. G. Rowntree for some indicators.

Since concentrations per liter and per kilogram may be readily converted one into the other if the density of the solution is known, charts for computing the density of sea-water are given in figures 1 and 2. This gives the actual density in question, as it is obviously insufficient merely to know the specific gravity at some standard temperature. The direct determination of the specific gravity by means of a floating hydrometer is often vitiated by large experimental errors, and many of these instruments are made for a standard temper-

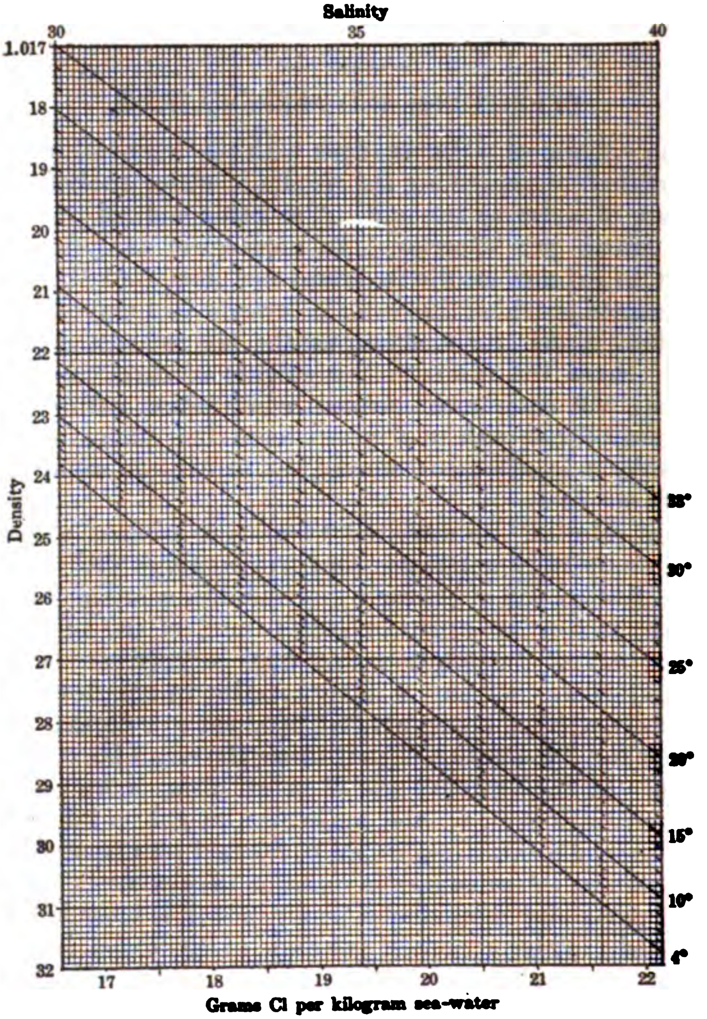


FIG. 1.—Conversion table for finding the density of sea-water (at any temperature), referred to distilled water at 4° from the salinity or the Cl in grams per kilogram. Each diagonal is for indicated temperature only.

ature on the obsolete Réaumur thermometer scale (14 R. = 17.5 C.), which is often unattainable aboard ship or at seaside laboratories. The best practice is to titrate the chlorides of the sea-water with silver-nitrate solution, using potassium chromate as indicator, and, if a constant temperature can not be maintained, to calibrate each new silver solution with standard sea-water. Since the temperature changes affect the volumes of the silver solution and sea-water about equally, they may be ignored. The concentration of sea-water was formerly expressed as the *salinity* or total salt-content, but it was found that the ratio of the chief salts in sea-water is remarkably constant, and the individual elements may be determined much more accurately than the total salts in one operation. Salinity is no longer determined directly, but is calculated from the chlorine titration according

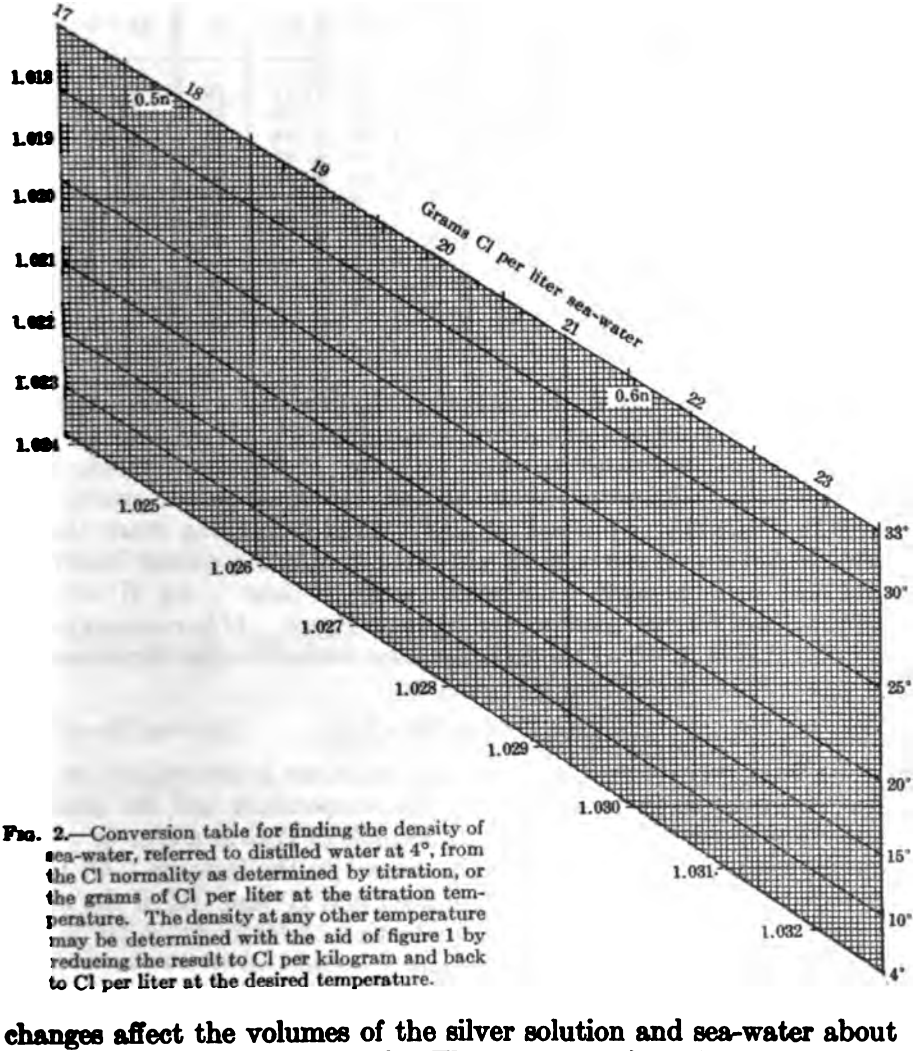


Fig. 2.—Conversion table for finding the density of sea-water, referred to distilled water at 4°, from the Cl normality as determined by titration, or the grams of Cl per liter at the titration temperature. The density at any other temperature may be determined with the aid of figure 1 by reducing the result to Cl per kilogram and back to Cl per liter at the desired temperature.

to the Sörensen-Knudsen definition. If it is desired to know the salinity (S) more accurately than can be determined with the aid of figure 1, the following conversion table (Knudsen) may be used:

(Integral part and decimal point omitted in table.)

Cl per kilo 19.37 g = S 35.00										19.93 g = 36.00 S	
Cl	S	Cl	S	Cl	S	Cl	S	Cl	S	Cl	S
37	00	48	19	59	39	70	59	81	79	93	00
38	01	49	21	60	41	71	61	82	81	94	02
39	03	50	23	61	43	72	62	83	82	95	04
40	05	51	25	62	44	73	64	84	84	96	06
41	07	52	26	63	46	74	66	85	86	97	08
42	08	53	28	64	48	75	68	86	88	98	09
43	10	54	30	65	50	76	70	87	90	99	11
44	12	55	32	66	52	77	71	88	91	20	36
45	14	56	34	67	53	78	73	89	93	00	13
46	16	57	35	68	55	79	75	90	95		
47	17	58	37	69	57	80	77	91	97	20.00 = 36.13	
								92	99		

As already mentioned, the unit of the hydrogen-ion concentration is 1 n $H^\cdot$, or about 1 gram of hydrogen ions per liter. The quantity actually found in sea-water is about 1 gram in 100,000,000 liters, or 0.000,000,01 n. In order to avoid the use of so much space, this number is usually expressed as 10^{-8}, which is nothing more than a minus logarithm to the base 10—in other words, a common logarithm with the sign changed. We might write it thus: $-\log H^\cdot = 8$, but Sörensen still further abbreviated it thus: $P_H = 8$. It is necessary only to remember that when the hydrogen-ion concentration decreases the P_H increases in the following manner:

0.1 n $H^\cdot = P_H 1$ 0.00000001 n $H^\cdot = P_H 8$ 10^{-14} n $H^\cdot = P_H 14$.

The H-ion concentration of aqueous solutions is determined by the dissociation of water as affected by the temperature and the presence of acids or bases. There are about 55 mols of H_2O in a liter of water, that is, its concentration is 55 m in respect to the formula. Of these 55 mols, only about 10^{-7} m is dissociated into $H^\cdot$ and OH' if the water is pure. In other words, the concentration of $H^\cdot \times OH'$ is 10^{-14}. If we add acid we increase the H ions and if we add alkali we increase the OH ions, but the concentration of $H^\cdot \times OH'$ remains constant ($= 10^{-14}$). This dissociation constant of water is abbreviated K_w, and hence $-\log K_w = 14$. Rise in temperature increases the dissociation of water; hence $-\log K_w$ decreases, being 14.07 at 20° and 13.73 at 30°, or a fall of 0.34; hence the P_H falls 0.17. We might expect the P_H of sea-water to fall the same amount as that of pure water with rise in temperature, but such is not the case. Rise in temperature causes increased hydrolysis of bicarbonates, and hence increases the OH-ion

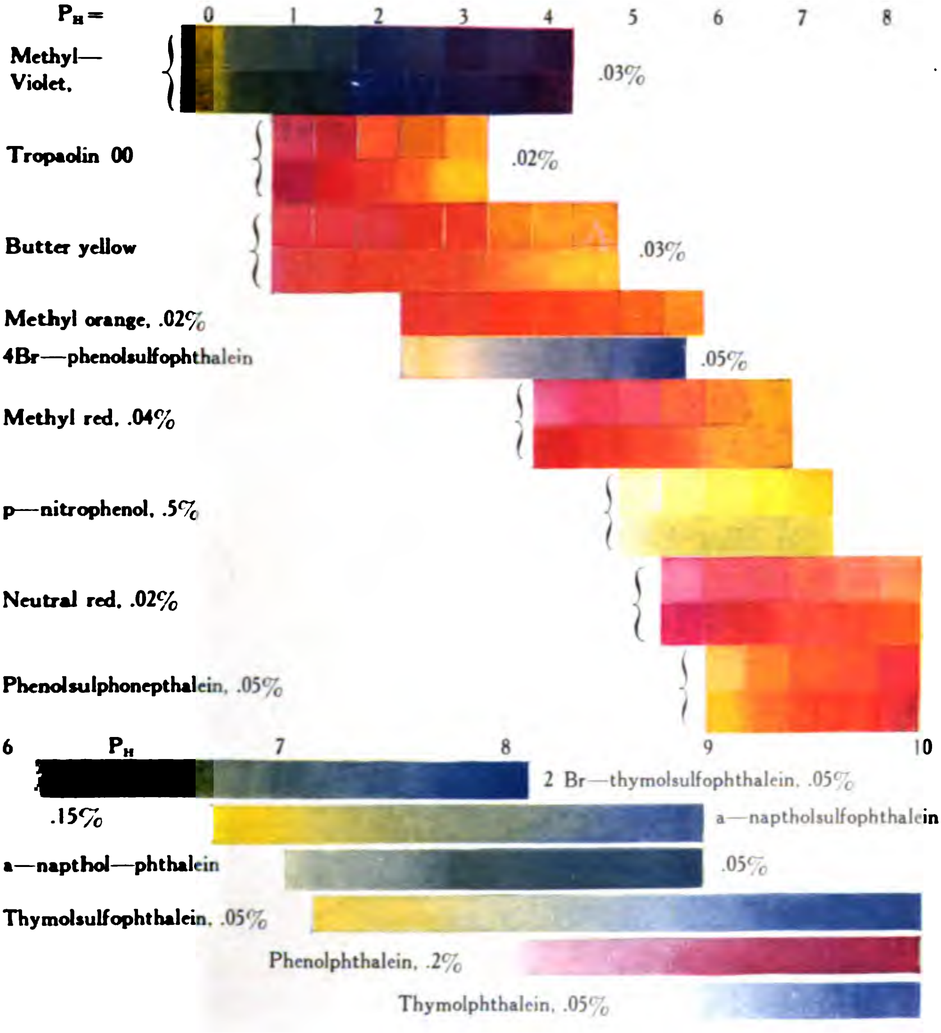


FIG. 3.—Conversion table for roughly determining the P_H of any aqueous solution. The solution is placed in a test-tube of 1 cm. bore together with 0.1 c.c. of the indicator solution of the indicated concentration and the color is compared with the chart while looking downward into the tube. In many cases two attempts to match the color were made, the continuous scale being made with the aid of water colors and the discontinuous scale with the aid of aniline dyes. About 0.3 must be subtracted from the P_H of sea-water as determined by this chart if sulfophthalein indicators are used, to correct for the salt error. For finer work the tubes described in this paper are recommended.

concentration, and these OH ions combine with the newly formed H ions to form water again. Any change in the H-ion concentration of sea-water due solely to change in temperature (*i. e.*, provided the temperature change is not allowed to cause a loss or gain of CO_2) is within the limits of error of our measurements. All measurements were made with the hydrogen electrode, which under proper conditions is affected only by the hydrogen ions. The indicators that were calibrated are weak acids and hence should be affected by H ions and not by OH ions. The indicators are affected by Na ions to a slight extent, as given in the calibration table. Temperature changes affect these indicators, but the ordinary changes in temperature affect them so slightly that it has not yet been possible to estimate the temperature correction. Furthermore, the temperature coefficient in sea-water and standard borax solution is probably the same.

The CO_2 tension of a solution is the fugacity or escaping tendency of CO_2. It is measured by estimating the partial pressure of CO_2 in an atmosphere in equilibrium with the solution—*i. e.*, the pressure of CO_2 required to prevent the escape of CO_2 from the solution. The air is not quite in equilibrium with the surface-water of the sea, so that it is not sufficient to determine the CO_2 partial pressure in the air unless it is shaken with sea-water. Since the average CO_2 in air is 3 parts in 10,000, the CO_2 pressure is given in parts per 10,000 of a standard atmosphere of 760 mm. Hg. The total CO_2 content of sea-water is the total amount of CO_2 that may be obtained from it by boiling or evacuating or aeration with indifferent gas after adding an excess of acid to decompose the carbonates. It is not in the form of a gas in sea-water, but exists in several molecular species: CO_2, H_2CO_3, HCO_3', CO_3'', and carbonates and bicarbonates of all of the bases present. The CO_2 is estimated as cubic centimeters of the dry gas at 0° and 760 mm., and may be reduced to milligrams by multiplying by 1.965.

The $H^{\cdot}$ concentration of sea-water is maintained fairly constant by the presence of salts of weak acids called buffers. These are, first CO_2 and second H_3BO_3, H_3PO_4, SiO_2, and As_2O_3, which are not lost by volatilization under conditions in the sea, and are collectively abbreviated to non-volatile buffers. The P_H of sea-water remains constant so long as these buffers (especially CO_2) are not increased or diminished in proportion with the bases combined with them.

RESULTS APPLIED TO OCEANOGRAPHY AND MEASUREMENT OF RESPIRATION-RATE OF MARINE ORGANISMS.

All of the P_H determinations were made with the hydrogen electrode, but at the same time the indicator method was calibrated so that the results may be duplicated by this more convenient method. The indicators used were mostly selected from the list shown in figure 3. It was found that the sulfophthalein series gave the most reliable results because of the brilliancy of the colors and the fact that they are differently colored in acid solutions and hence the change is qualitative and not quantitative. An error due to a difference in concentration of the indicator may be at once observed, whereas this is not possible with phenolphthalein. Dibrom-o-cresolsulfophthalein has the same range as p-nitrophenol; dibromthymolsulfophthalein has the same range as phenolsulfophthalein; dibromthymolsulfophthalein has about the same range as a-naptholsulfophthalein.

Part of the work was done at Tortugas, Florida, and the sea-water was drawn directly from the sea into the apparatus, but owing to the inability to work at any temperature except 30° and also to the incompleteness of the library, the work could not be finished there, at least in one summer. Part of the results obtained at Tortugas have already been published (McClendon, 1916).

The work was brought to the present state of completeness in Minneapolis, and the experiments at 30° are in close agreement with those made at Tortugas. The sea-water that had been used in aquaria, even those of pyrex glass, was deficient in non-volatile buffer, due to the action of organisms, and could not be used in standard experiments. In all of the Minneapolis experiments one of the sulfophthalein indicators was added to the extent of 1 mg. to 100 c.c. of sea-water, and so increased the non-volatile buffer to this slight extent; this causes a small theoretical error, but was necessary in order to make possible the duplication of the experiments by the indicator method. There was not sufficient time for another series of experiments without the indicator, as the sea is too complex a solution to be treated in any simple mathematical manner, and it was necessary to make a large number of direct determinations over a wide range of conditions. Some of the determinations were repeated from 2 to 10 times before we were confident of the correctness of the results. During the investigation the solution of o-cresolsulfophthalein was accidentally lost and a new one was prepared in the same manner and from a bottle of the same label by the same manufacturer. It was found that a buffer mixture containing this indicator had the same color as a buffer mixture of 0.15 higher P_H colored with the old solution. This led to a repetition of much of the work. It has long been known that such an experience may be had with any indicator, but it is hoped that these excellent indicators may be manufactured in larger quantities, so as to insure greater uniformity, and that each yield be numbered and so labeled.

Alkaline solutions of borax and other buffer mixtures of standard P_H are affected by absorption of CO_2 by the air and solution of glass. Those sealed in 1 cm. nonsol glass tubes were found to keep for about a year. In order to increase their life and accuracy, new solutions of higher buffer value were standardized and sealed (by fusing the glass) in 24 mm. nonsol tubes containing the indicator. These tubes should be good for 5 years, provided they are kept in the dark most of the time.[1]

Details of the indicator method and the colorimeter that we used are given on pages 44–46, and we will now pass on to the question of the duplication of our results by the indicator method.

We found the P_H of sea-water to be practically independent of salinity or temperature between 20° and 30°, and all of our experiments at 10° indicate that even this drop in temperature does not appreciably affect the P_H. Our values given for 0° are extrapolated from CO_2 data on the assumption that the P_H is independent of the temperature.

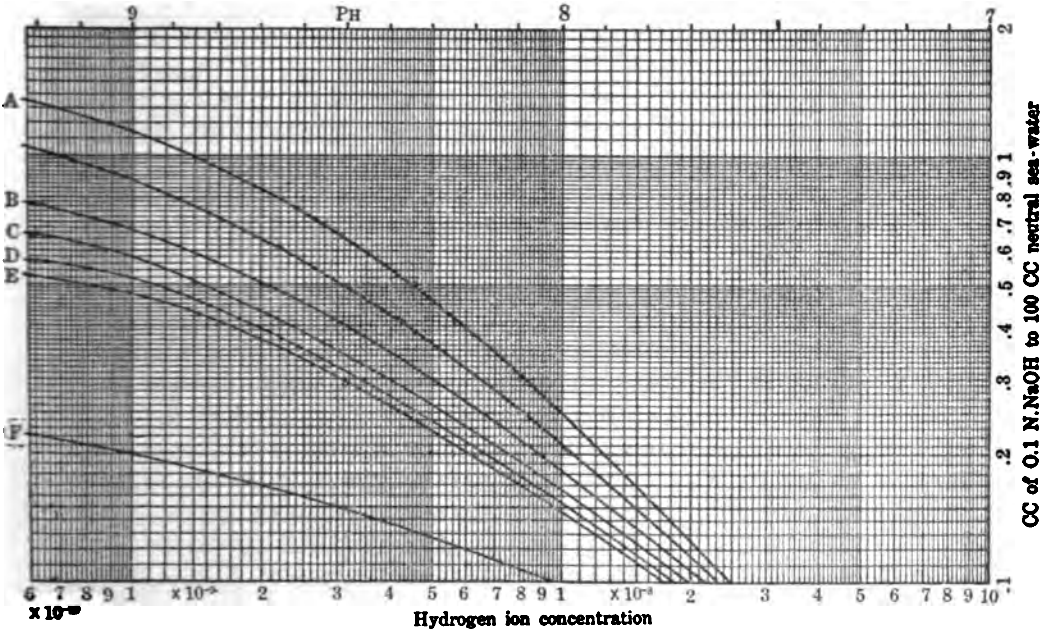


FIG. 4.—Results of electrometric titration of CO_2-free sea-water plus indicator with CO_2-free NaOH solution. A very slight excess of HCl is added to the water and the CO_2 driven off by boiling, the water is brought about to the neutral point ($P_H = 7$), and then titrated. On the ordinate is measured the logarithm of the number of cubic centimeters of 0.1 normal NaOH added to 100 c.c. of the neutral, CO_2-free sea-water, and on the abscissa is measured the P_H. At the bottom is a scale for conversion of the P_H into normality of H-ion concentration. The titrations were made on sea-water from the following sources: *A*, artificial sea-water containing 0.0015 molecular boric acid. *B*, sea-water from Tortugas, Florida. *C*, artificial sea-water containing 0.0008 molecular boric acid. *D*, Tortugas sea-water in which diatoms and other Protista had been grown in a pyrex flask for about 6 months. *E*, sea-water collected somewhere off Hatteras and kept in the Princeton marine aquarium about 6 years, the evaporation being compensated by addition of rain-water. *F*, artificial sea-water without any non-volatile buffer. Later experiments showed these data to be merely relative.

We found the P_H of sea-water to be determined solely by ratio of the concentration of buffers, including CO_2 and other weak acids, to the concentration of bases combined with them (excess base over strong acid).

---

[1] Duplicates of our tubes and colorimeter may be obtained from the manufacturers of the indicators.

Attempts were made at Tortugas and continued at Minneapolis to estimate the non-volatile buffers separately, and to find some reliable data in the literature, but this work has not reached a state for publication. It was found that phosphates and silicates are soluble enough, but it seems impossible to find enough silicate and phosphate in sea-water to account for all of the non-volatile buffers, even though we regard the results of analyses as being rough estimates. Borates were found in Tortugas sea-water and in the Princeton Marine Aquarium and in the Pacific Ocean by the senior author. An attempt was made to increase the non-volatile buffer by the addition of phosphates, but marine animals behaved abnormally in this water. The same was attempted with silicates, and plants grew luxuriantly in the artificial sea-water. No more accurate data were found when the Tortugas paper was sent to press (the paper was received for publication September 14), but about 6 weeks later the paper of Henderson and Cohn appeared, in which it was stated that artificial sea-water containing boron equivalent to 0.0015 boric acid had the same buffer value as sea-water. This led us to make a series of electrometric titrations, continuing those begun at Tortugas, on a series of natural and artificial sea-waters under CO_2-free conditions. The results are given in figure 4, and it was estimated from this and tonometer experiments that Tortugas sea-water has a non-volatile buffer value much less than 0.001 m boric acid, whereas by the growth of diatoms and other marine Protista in a pyrex flask the same water was reduced to less than this; and Princeton aquarium water, kindly sent by Dr. L. R. Cary, had a still lower non-volatile buffer value. It is not possible to determine the exact boric-acid equivalent from figure 4, and in estimating that of Tortugas water the P_H of artificial sea-waters and Tortugas sea-water at the same CO_2 tension were found to be about equal.

We have not been able to obtain a sample of sea-water with as high non-volatile buffer value as artificial sea-water of 0.0015 m boric-acid content. Veatch reported evidence that the shore-water off the southern California coast is in communication with unknown borax deposits. Dr. William E. Ritter kindly sent us a sample of this water, and it was found to give the same qualitative test for boric acid as any of the other sea-waters examined. It had a non-volatile buffer value equivalent to Tortugas sea-water. We feel justified, therefore, in the assumption that the non-volatile buffer value of sea-water of fairly normal salinity is a more or less constant quantity and is markedly changed only when organisms are kept a long time in a relatively small quantity of sea-water. The water at the surface of the ocean, where most of the organisms live, is constantly being renewed by vertical ocean-currents.

On the assumption that the weak acids in the sea have a somewhat comparable buffer value, the error that might arise in calculating the

CO_2 content from the P_H could be large only in case variation in non-volatile buffer is large. But CO_2 is more than 100 times as strong an acid as boric acid, the dissociation constant of the former being about $10^{-6.5}$ and of the latter $10^{-9.25}$. CO_2 may neutralize 4 times as much NaOH as boric acid is able to, the formulæ being Na_2CO_3 and $Na_2B_4O_7$ (although the solution is not neutral in either case, being alkaline, due to hydrolysis). We may assume therefore, that the maximum error in estimating total CO_2 from P_H, due to variation in the non-volatile buffer, is negligible (not including any other sources of error).

The estimation of the excess base is as follows: Owing to the presence of boric and traces of phosphoric acid phenolphthalein can not be used, since we have found that it gives a result about 5 to 10 per cent too low. Methyl red is not as sensitive and may give too high a result, and it is better to use the same indicator we used if comparative results are desired. 100 c.c. of sea-water are placed in an Erlenmeyer flask of resistance glass with etched mark at 100 c.c. and enough dibrom-o-cresolsulfophthalein solution to color it a distinct purple; then enough 0.01 n HCl is run in from a burette to turn it yellow. The flask, together with a resistance-glass or quartz beaker of distilled water, is boiled, preferably on an electric hot plate (alcohol flame is better than gas if electricity is not available). The sea-water will become purple again, and more acid should be added to turn it yellow and the boiling continued. Wooden tongs or a wooden model of a nut-cracker are preferable to a wire test-tube holder for handling the hot flask. The end-point is reached when just enough acid has been added to turn it yellow, and it does not turn purple on further boiling for 5 minutes, while the volume is kept approximately constant by additions of the boiling distilled water. Distilled water that has remained a long time in soft glass should not be used, and if water free from solid residue can not be obtained, it is better not to restore the volume, but to repeat the titration with the addition of all but 1 c.c. of acid before boiling and thus accelerate the evolution of CO_2. Prolonged boiling after the end-point has been reached is to be avoided, owing to the slight solubility of even the best resistance glass. It is theoretically possible to boil off traces of HCl after the end-point has been reached, but this process is necessarily extremely slow at so low an acidity. Normal sea-water will require a little more or less than 25 c.c. of 0.01 m HCl per 100 c.c. or 25 c.c. of 0.1 n HCl per liter, and this number (for example 25) is used to denote the excess base over strong acid (*i. e.*, the base combined with the buffer acids).

In titrating the non-volatile buffers the same procedure minus the indicator is followed (though it is possible to cut all of the quantities in half if necessary). The entire quantity of acid is added at first and the sea-water boiled about 15 minutes gently, in order to remove all of the CO_2. Any CO_2 in the water will be estimated as non-volatile buffer and influence the result. The equivalent of about 1 c.c. of 0.1 n NaOH is added to the boiling sea-water; the volume is quickly and accurately restored with boiling distilled water, and the flask tightly closed with a rubber stopper. The NaOH solution must be free from CO_2 and contain little silicate. The method of its preparation and protection is given on page 45. The exact quantity of NaOH to be added can not be stated, but there should not be sufficient to cause precipitation of earthy hydroxides. It is best to have two series of tubes of known P_H, one colored with thymolsulfophthalein and the other with either o-cresolsulfophthalein or a-naphtholsulfophthalein—in other words, a total range of $P_H = 7.5$ to 9 or greater, but only the first series of tubes are necessary. Two nonsol test-tubes of exactly the same bore as the standard tubes and with etched marks at the volume to be titrated are provided, each with a rubber stopper with a central perforation closed with a glass rod. The required quantity of indicator is placed in each of the tubes and sea-water from the flask poured in (to the mark) and the stopper quickly inserted. Care must be taken never to breathe toward the test-tube unless it is tightly closed. The tube with thymolsulfophthalein is first examined and compared with the standard tubes and its P_H recorded. The glass rod is removed from time to time and small quantities of 0.1 n HCl are run in from the burette. The tube is shaken and its P_H is recorded together with the burette reading.

This process is repeated until the limit of the indicator is nearly reached, when enough indicator is added to correct for the increased volume and any error due to dilution of the indicator is recorded. The same process is repeated with the other indicator and the results are compared with figure 4.

If the excess base is known the total CO_2 may be determined from the P_H by using the conversion table in figure 5. The three diagonal lines are for sea-waters containing 23, 24, and 25 excess base respectively. The smallest possible difference in CO_2 per liter that we have been able to determine by means of the standard tubes is 0.1 c.c. but in order to do this it is necessary to have a finer gradation of tubes than are ordinarily used. Such a degree of accuracy is only relative since the absolute accuracy of the conversion table probably does not exceed 1 per cent. The diagonals in the conversion table are drawn as straight lines, but this is merely an approximation. If they were extended they would have to be curved or lose in accuracy. The sea is of too complex a composition to admit of any simple mathematical relations. Empirical formulæ, such as the one developed by Fox for estimating the total CO_2, may be discovered, but they are only approximations. Exact data may be obtained only by direct experiment, and the result depends on the technique.

If the sea-water contains more than normal non-volatile buffer, the CO_2 content will be less than that read from the table, and *vice versa*, but the quantitative side of this relation has not been thoroughly investigated. We may picture it in the following manner: Suppose the water is sealed in an air-tight container so that CO_2 can not escape. If we add boric acid part of it will remain free and increase the number of H ions; but since it is so weak an acid, this change will be slight. Part of the boric acid will displace CO_2 from bicarbonates and some of the CO_2 will dissociate and increase the number of H ions still more. Since boric acid is weaker than CO_2 it will not decrease the P_H as much as the same number of molecules of CO_2; hence the error in estimating the CO_2 will not be as great as the same molecular concentration of boric acid. SiO_2 would have even less effect than boric acid, but phosphoric acid would have a relatively greater effect. We know of no determinations of As_2O_3 in sea-water, but Bang found 4 mg. per kilogram of fish.

The conversion table in figure 5 has a very limited range, and the further it is extended graphically the greater the error. We did this for the Princeton aquarium water because it had the very abnormal excess base of about 44.5. We determined the total CO_2 and P_H on a portion of it and Dr. L. R. Cary determined the P_H of another portion and the change in P_H on further additions of CO_2. The results are:

Total CO_2 from experiment.	Total CO_2 from table.	Difference.	P_H
88.4	82.2	− 6.2	8.1
99.4	100.0	+ 0.6	7.5
114.8	115.2	+ 0.4	7.0
143.7	127.5	−16.2	6.6

The difference of 6.2 c.c. CO_2 at P_H 8.1 is only 0.0004 m, but the difference at P_H 6.6 is probably partly due to an error in the extended table or the experimental data. It may be remarked that the history of this abnormal water does not completely solve the question of the origin of the difference in excess base. It is possible that some concrete may have been dissolved, although it was said to have been paraffined. Concrete may possibly have been dissolved by the rain-water used to compensate for evaporation, or limestone dust may have gotten into the rain-water by being blown onto the roofs. These characteristics of the water were not investigated when it was first received.

In using the conversion table in figure 5 for the study of the respiration of marine organisms, only relative values are necessary, and it is thought wholly adequate for the purpose. It would be of little advantage to extend the table to a greater P_H range unless it is first absolutely established that the abnormal P_H does not make the organisms physiologically abnormal and that oxygen is still present in the water (see oxygen scale at left of figure 5 for temperate and tropical seas). Th scale at the extreme left shows that the temperature is raised only 0.04 by animal respiration that uses up all of the oxygen in the warme seas. This shows the impossibility of the existence of a warm-bloode animal with gill respiration.

The oxygen-content of sea-water may be estimated from the P_H since the differences between cubic centimeters of CO_2 and O_2 in samples of water of the same excess base are reciprocal.

The change in P_H with change in CO_2 tension is affected only by th temperature, as shown in conversion table in figure 6.

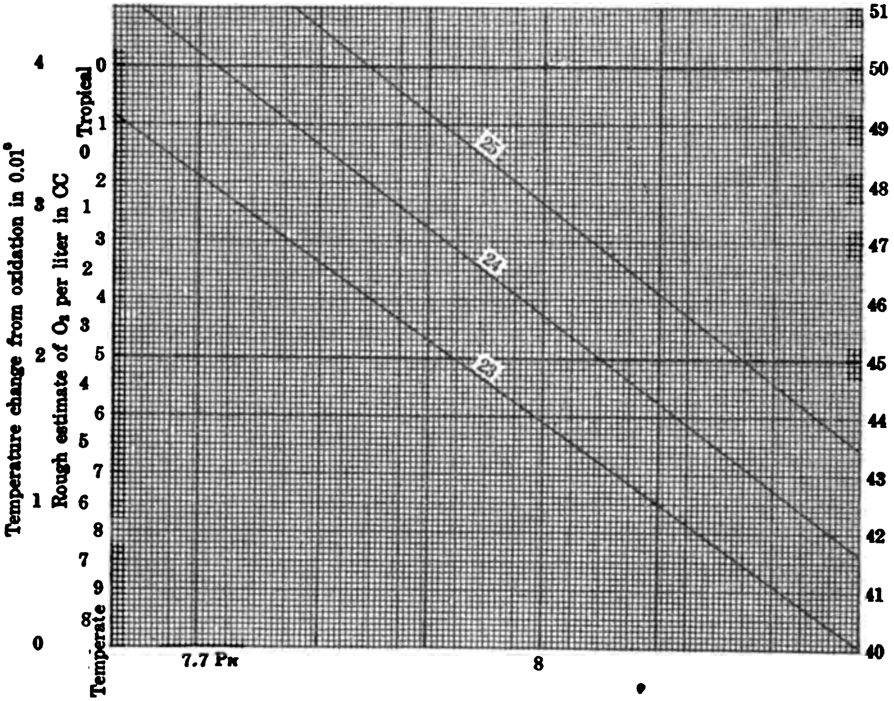


FIG. 5.—Conversion table for determining the total CO_2 and O_2, and the rise in temperature sea-water due to the respiration of marine animals, from the excess base and P_H. The thr diagonals are for three values of the excess base. On the abscissa is given the P_H and on tl ordinate are four scales, one for the total CO_2, one for the approximate O_2 of tropical ocean one for the O_2 of temperate oceans of about 20°, and one for the approximate rise in temper ture due to respiration of marine animals, in hundredths of a degree. This estimate of rise temperature is a little too small, but can not be calculated exactly.

Changes in salinity theoretically affect the CO_2 tension and henc CO_2 content of sea-water. Fox divides the CO_2 into two parts, one c which obeys Henry's law and never exceeds 1 per cent of the total It is this small fraction that is affected by salinity, but the changes i salinity ordinarily met with in the larger oceans change this fractio only about 1.5 per cent, and the total change is exceedingly small We have not been able to determine any effect of salinity on P_H or CC content.

Henderson and Cohn state that if the CO_2 tension is maintained constant (at about 0.0004 atmosphere) the P_H changes from 8.03 at 30.73 salinity to 8.1 at 34.6 S. We were unable to confirm this result with the hydrogen electrode. Henderson and Cohn used the indicator method of Sörensen and Palitzsch, and the difference between successive standard tubes as recorded in several of the papers of these authors is $P_H = 0.1$. The tubes were exposed to the air, but were occasionally renewed. Their results were sometimes expressed in figures of four decimal places, but this probably was not intended to have more than mathematical significance. In fact, we found that, with the most accurately measured sealed tubes placed in a colorimeter that brings the centers of the tubes together on a sharp line, we could not distinguish with certainty any difference smaller than about P_H 0.02, even in the most sensitive region of the range of the most brilliant indicators. The fact that Henderson and Cohn observed P_H 8.06 at S 31.58, 8.07 at 32.05, and 8.06 at 32.45, indicates that they made small errors in the estimation of P_H or CO_2 tension or temperature. Sörensen and Palitzsch give the salt error of their indicators at 20 and at 35 salinity, and Henderson and Cohn may possibly have attempted to estimate the change in salt error from S 30.7 to 34.6, but they do not mention the fact.

We have recalibrated our salt errors about 50 times, but the different determinations do not coincide within an error of less than 0.025 P_H. On the average it was found to be necessary to subtract 0.05 from the observed indicator P_H (sulfophthalein series) when the salinity was increased from 30 to 37.7 in order to obtain the same result as with the hydrogen electrode. If this correction is applicable to Henderson and Cohn's indicator for this range (probably phenolphthalein) the increase in P_H with rise in salinity from 31.58 to 34.6 is reduced to 0.2 and is about the limit of the smallest possible difference that can be determined with the eye, whereas the range of salinity is as great as is ordinarily met with in the larger oceans. We feel safe, therefore, in assuming that the influence of salinity on the relation of CO_2 tension to P_H at any specified temperature in negligible. In fact, Henderson and Cohn do not state the variation in temperature. In our experiments the temperature was observed within 0.05° and controlled within 0.2° during the majority of the experiments and to within 0.1° during critical periods.

In figure 6, the curves for 30°, 20°, and 10° are taken from the means of many determinations, whereas the curve for 0° was extrapolated. The results of Henderson and Cohn's experiments at 20° are marked by crosses. Their results at higher CO_2 tensions more nearly fall on a smooth curve, but it is impossible to compare them with hydrogen-electrode determinations, because the hydrogen electrode is not very reliable in low partial pressures of hydrogen, even though a correction be applied for change in hydrogen pressure or concentration.

By means of the conversion table (fig. 6) it is possible to determine th CO_2 tension of sea-water from the temperature and P_H as read by mean of the standard tubes and colorimeter described on pages 44–46. Th

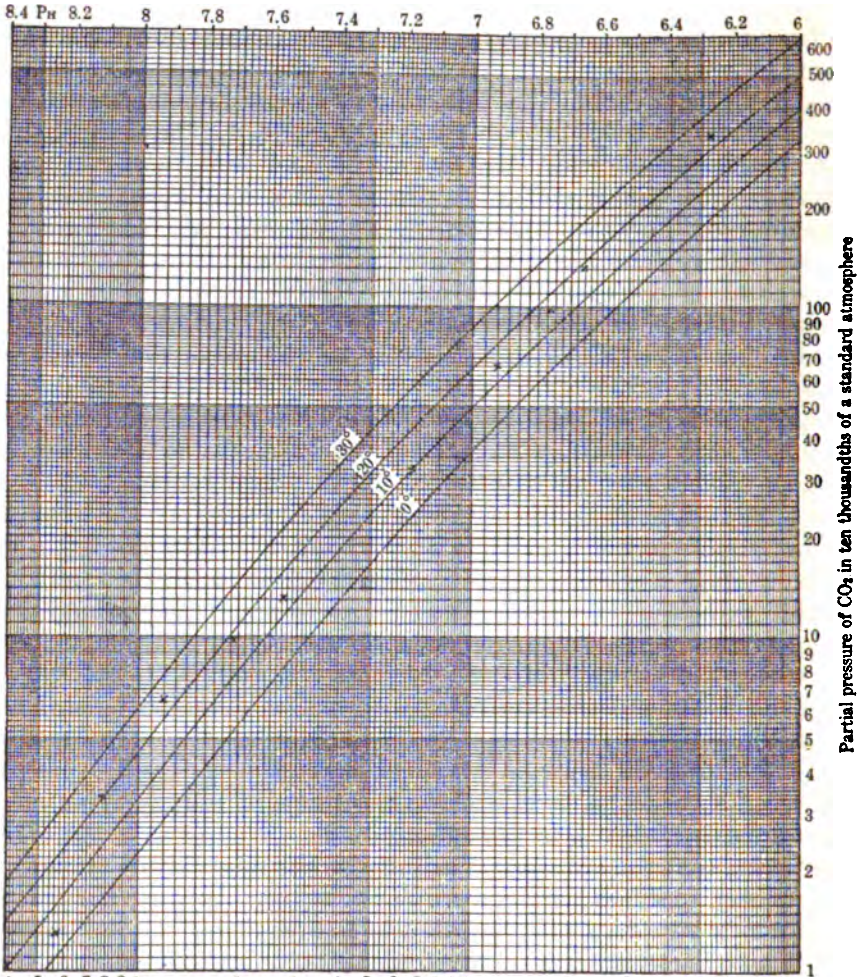


Fig. 6.—Conversion table for finding the CO_2 tension of sea-water from the P_H and the temperature. On the ordinate is measured the logarithm of the CO_2 tension in ten-thousandths of an atmosphere. On the abscissa is measured the P_H, with a scale below for converting it into th normality of H ions. The four curves are for 30°, 20°, 10°, and 0°, the last of which has been extrapolated. The crosses mark the determinations of Henderson and Cohn by the colorimetric method at 20°.

CO_2 tension is of first importance in the respiration of marine organisms. The rate of elimination of CO_2 by animals and its absorption by plants in the sunlight is directly proportional to the difference in CO_2 tension just outside and inside of the respiratory surfaces. Life in the sea is more abundant in regions that are vigorously stirred, so that the CO_2 tension of a sample of the sea-water may be considered almost equal to that of the sea-water bathing the respiratory surfaces. The CO_2 tension has been one of the most difficult characteristics of sea-water to determine accurately.

It has been supposed that the amount of CO_2 in sea-water regulates the growth of seaweed, but the reverse is probably more nearly correct. The respiratory quotient of marine organisms seems to be about 0.7 to 1.0 and the respiration of animals and plants reciprocal. Some marine bacteria take their oxygen from nitrates, but this effect must be minute, since the supply of nitrates is small. The atmosphere can not be the chief regulator of the CO_2 of the sea, since there is about 30 times as much CO_2 in the sea as in the air. There is always a superabundance of CO_2 in sea-water to supply the needs of green, red, or brown seaweed, but by using it the plants increase the P_H of the water. It seems probable that the plants grow rapidly until the P_H that is most favorable to them is exceeded. This is in harmony with the fact that the P_H of the great oceans to the depth penetrated by light is more constant than the CO_2 tension, the P_H varying from about 8.0 to 8.25 and the CO_2 tension from about 0.00015 to 0.0005 atmosphere. The sea may be compared to the body of one of the higher vertebrates. The mammal regulates the P_H of the blood through the action of the respiratory center. The sea regulates the P_H of its surface-water most probably through the action of seaweed. The limit in the supply of oxygen probably prevents animal life from getting the upper hand temporarily and thus endangering the communal life in the sea.

It seems probable that seaweeds regulate the CO_2 of the atmosphere. The gaseous exchange between sea and air is necessarily at the surface and is comparatively slow. Bohr observed that the absorption of CO_2 from an atmosphere of the pure gas by CO_2-free water that is stirred (probably more vigorously than the sea ever is) is about 0.1 c.c. per square centimeter of surface per minute. Since the difference in CO_2 tension between air and sea seems never to exceed 0.02 per cent of that in Bohr's experiment, except in the polar regions, the rate of diffusion would not exceed 0.00001 c.c. per square centimeter per minute or 0.1 c.c. per square meter per minute in a storm, and necessarily much less in calm weather on account of the lessened rate of stirring at the surface. When we consider the volume of the sea and air compared to the sea-air surface, the fact becomes intelligible that the CO_2 in the air is relatively constant (3 per 10,000) in the different regions of the world where it has been accurately measured, whereas the CO_2 tension of the sea-surface varies from 1.5 to 5 per 10,000. The air is stirred more rapidly than the sea, and the CO_2 of the air seems to be determined by an equilibrium between gain in CO_2 over some regions of the sea surface and loss over others. The partial pressure of CO_2 in the air is therefore the average CO_2 tension of the sea-surface. The burning of a billion tons of coal per year is probably changing the CO content of the sea and of rocks and not of the atmosphere.

Regnard kept a tall open tube filled with a solution that becam colored in the presence of oxygen at constant temperature to preven convection currents. At the end of one year oxygen had diffused meters deep into the solution. Although some oxygen was used i coloring the indicator, the experiment illustrates the slowness of diffusion in liquids.

Since both the conversion table for CO_2 content and the one fo CO_2 tension are based on the assumption that the non-volatile buffe and the excess base in sea-water are constant, further work is bein done on this subject. It seems probable that the weak bases in sea water may assist the buffer action. The concentration of NH_3 i small, but is added to the total buffer value; it is non-volatile under th conditions present at the surface of the sea and is constantly bein replenished by rain as it is used by organisms. Aluminium can act bot as weak base and weak acid, but probably acts as a weak base in the sea Organic acids besides CO_2 may be classed as non-volatile buffers Their destruction in warm seas is probably due to the action o denitrifying bacteria, which catalyze the oxidation of organic matte with nitric and nitrous acids, as shown by Drew. The concentration o organic acids is probably maintained just below that which can b appreciably utilized by these bacteria.

The following artificial sea-water was found excellent for the growt of marine Protista and experiments in places where sea-water can no be obtained (the volumes being correct for 20°):

	Per liter.	m solutions.	Isotonic solutions.			Per liter.	m solutions.
	grams.	*c.c.*	*c.c.*			*grams.*	*c.c.*
$CaCl_2$, dry..	1.22	11.	(0.38 m)	29.0	Na_2SiO_3....	0.0025	....
$MgCl_2\cdot6aq$..	5.105	25.16	(0.37 m)	67.9	$Na_2Si_4O_9$...	....	0.005
$MgSO\cdot7_4aq$..	7.035	28.55	(0.975 m)	29.5	H_3PO_4.....	....	0.002
KCl........	0.763	10.23	(0.577 m)	17.7	H_3BO_3.....	0.062	1.
NaCl.......	282.7	483.65	(0.568 m)	852.0	$Al_2Cl_6\cdot12aq$	0.026	0.01
$NaBr\cdot2aq$...	0.0824	0.8	(0.565 m)	1.4	NH_3.......	....	0.001
$NaHCO_3$....	0.21	2.5	(0.930 m)	2.7	$LiNO_3$.....	0.0014	0.002
					H_2O.......	(to 1 liter)	373.63

If Na_2SiO_2 is used, it should be dissolved in the H_2O before addin the other salts or it will be impossible to get into solution. We use a thick sirupy solution of water-glass (said to be $Na_2Si_4O_9$) and mad an analysis of the Si concentration because we wished to ascertain th buffer value, but for the growth of organisms it is sufficient to conside the commercial solution as equivalent to 6 m SiO_2 or 1.5 m Na_2Si_4O and the quantity desired should be obtained by successive dilutions as a strong solution will precipitate when added to the other salts

For plants at least it is permissible to add a drop of the strong solution per liter and allow it to precipitate. If cultures are grown in soft glass, no further additions of Si will be necessary to supply that used up by diatoms.

When sea-water is evaporated and redissolved oxides of manganese and iron, $CaSO_4$, SiO_2, $Ca_3(PO_4)_2$, $CaCO_3$, CaF_2, $Ca(BO_2)_2$, Al_2O_3, $Ba(BO_2)_2$, $BaSO_4$, and, under certain conditions, $MgNH_4PO_4$ are difficult to get into solution, but by adding HCl and evaporating, a paste containing gypsum will be obtained which will go into solution in about 3 days and may be neutralized and 0.21 gram $NaHCO_3$ per liter added.

Another method of determining the CO_2 tension on board ship or in laboratories without general chemical equipment was developed. The portable micro-apparatus for determining the total CO_2, described in section 4, was used and conversion tables were prepared for determining the CO_2 tension from the total CO_2, the temperature, and the excess base. Figures 7, 8, and 9 are conversion tables for finding the CO_2 tension of sea-water of 23, 24, and 25 excess base, respectively. Each curve is for the indicated temperature only, and the values for 0° were extrapolated. Tables for sea-water of any other excess base titration may be plotted with the aid of figure 10, which shows the change of CO_2 with change in excess base when other factors are constant.

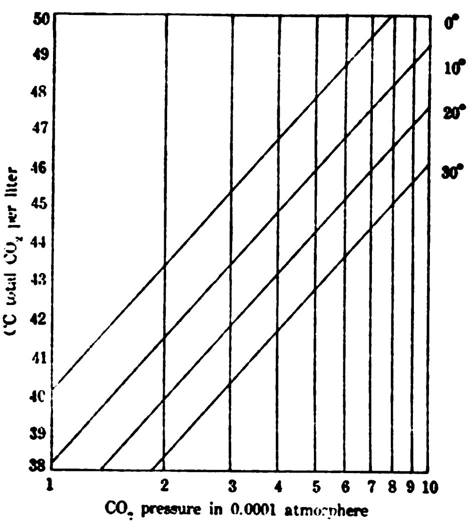


FIG. 7.—Conversion table for finding the CO_2 tension of sea-water of 23 excess base from the temperature and total CO_2. Each curve is for only one temperature, and that for 0° has been extrapolated. On the ordinate is given the total CO_2 and on the abscissa the CO_2 tension in ten-thousandths of an atmosphere.

Although it is theoretically a simple matter to determine the CO_2 tension directly, in practice it has been most difficult and attended with large experimental errors. Perhaps the most direct method is to shake a large quantity of the sea-water with a small quantity of air in a bottle until equilibrium is established, and analyze the air in the bottle. Errors arise in failure to reach equilibrium or to correctly determine the pressure and temperature or to correctly analyze the air. The analysis of one part of CO_2 in 10,000 parts of air may be done by the titration method, in which there are solubility and other errors, or by measuring the gas-volume contraction after absorption of CO_2, in which there are temperature, pressure, and meniscus errors. A conversion table was made by Fox. For those who may be confused at first by logarithmic paper the relation of P_H and CO_2 tension is shown on ordinary coordinate paper in figure 11.

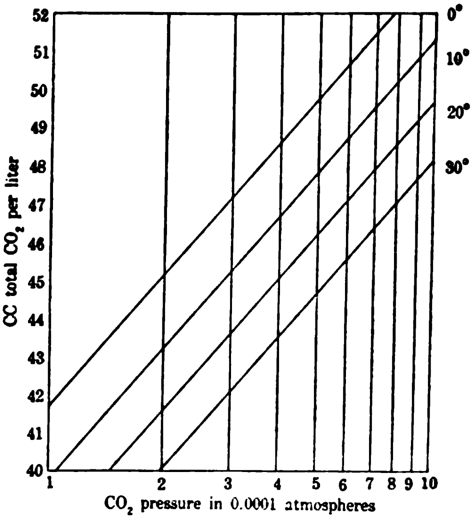


FIG. 8.—Conversion table for finding the CO_2 tension of sea-water of 24 excess base from the temperature and total CO_2.

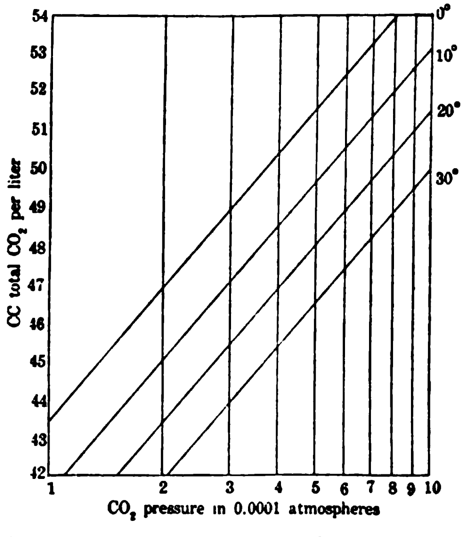


FIG. 9.—Conversion table for finding the CO_2 tension of sea-water of 25 excess base from the temperature and total CO_2.

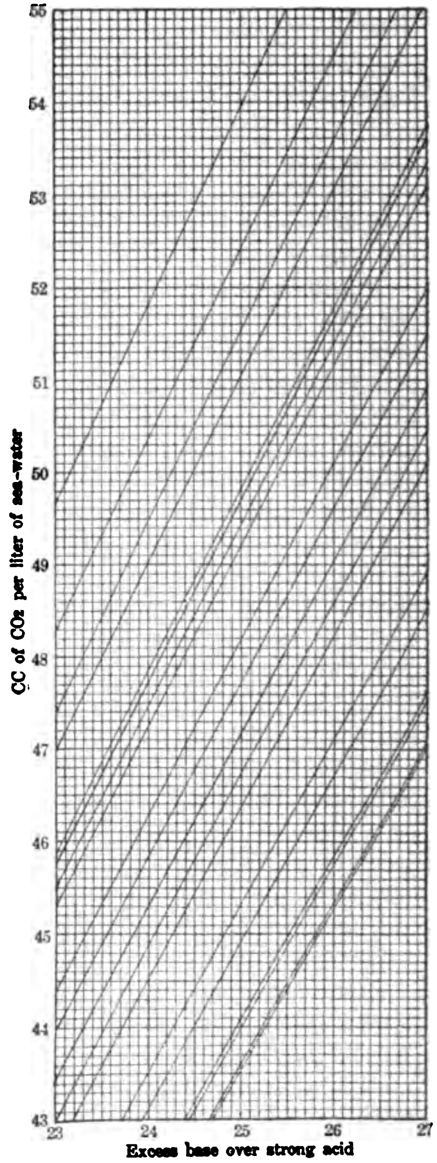


FIG. 10.—Data to be used in making a conversion table like fig. 9, but of any other excess base. Transfer values from fig. 9 to the 25 ordinate and draw diagonals through them parallel to the nearest diagonal in fig. 11; the values sought are found where these diagonals cut the ordinate of the excess base sought.

The determination of CO_2 of the air is made by placing about 10 c.c. of natural or artificial sea-water containing the thymolsulfophthalein in a nonsol test-tube of exactly the same bore as the sealed tubes and bubbling air through it to equilibrium and comparing with the tubes. The CO_2 tension of the air is read from figure 6. In order to hasten equilibrium the test-tube should be tall and have a series of constrictions above the portion necessary for colorimetry. A rubber stopper with 3 holes is inserted and a glass tube leading to the bottom, its closed end being pierced by fine holes; a tube is connected to a suction pump and a mercury manometer inserted in the third hole. The CO_2 content of the air is the CO_2 tension × 760 ÷ (the barometric reading — manometer reading). Thymolsulfophthalein is decolorized by bubbling air through its solution for a long time, but this seems not to be an oxidation or a reduction.

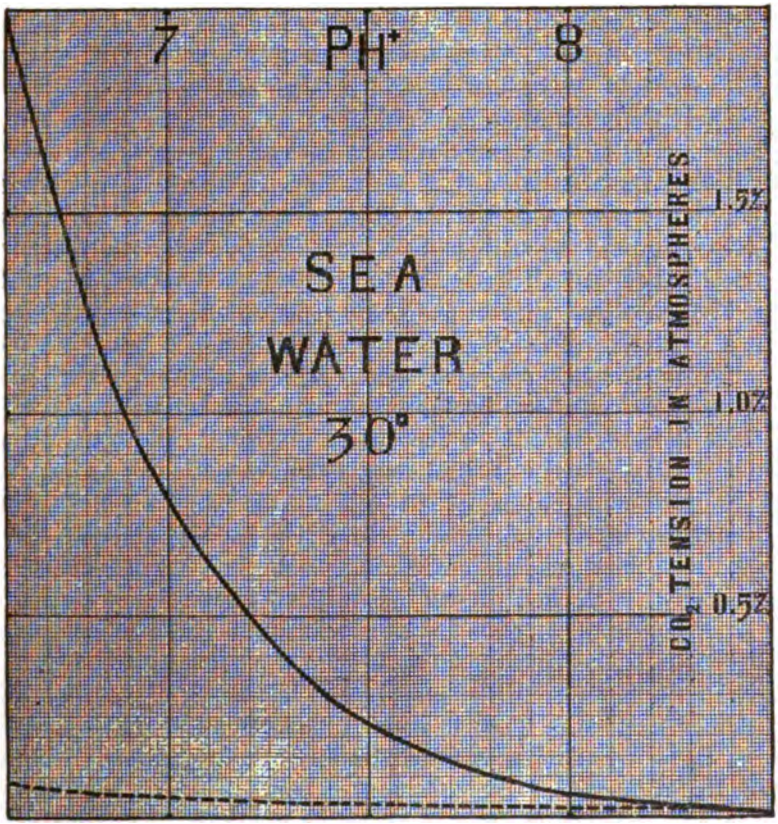


FIG. 11.—Conversion table for finding the CO_2 tension of sea-water at 30° from the P_H. This figure is explanatory of figure 6. The broken line is to be disregarded. (From Jour. Biol. Chem., 1916, XXVIII, 135.)

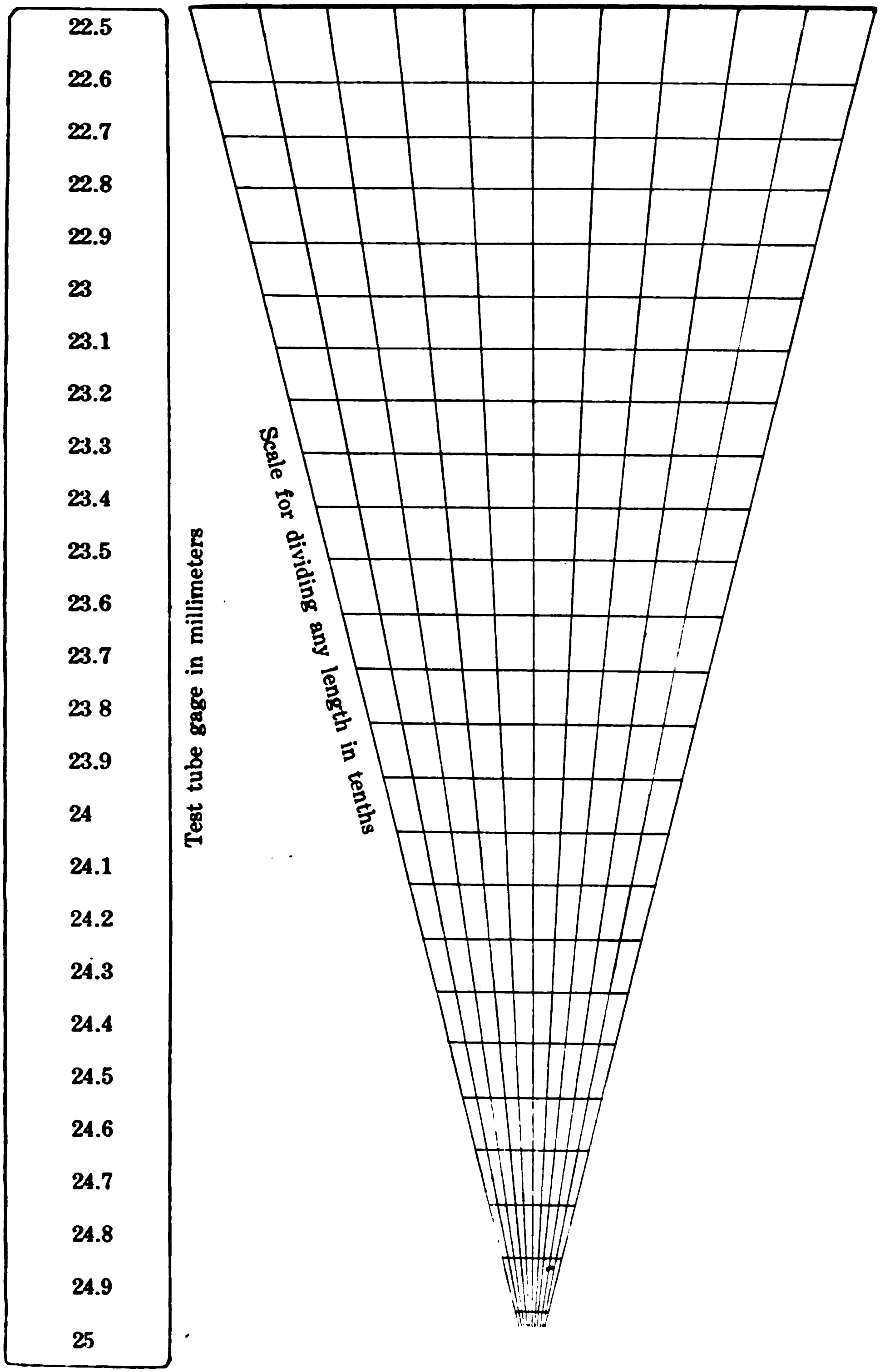
CALIBRATION OF AN IMPROVED INDICATOR METHOD FOR USE SHIPBOARD AND IN POORLY EQUIPPED LABORATORIES.

After the excellent work of Sörensen and his associates on indicator method it might be supposed that the last word had t spoken on this subject. But there are several disadvantages Sörensen's method. The solutions have to be exposed to the air du the determinations and are placed in such a position that the operat breath might blow directly into them. (This has been obviatec our method by sealing the tubes by fusing the glass, as done by Ro tree and his associates.) The salt-content of Sörensen's buffer mixtu is much less than in sea-water, and a large salt correction had to applied, and no new indicators could be used until their salt error determined. This has been obviated by calibrating new buffer n tures of the same salt-content as sea-water. The use of a colorime that brings the centers of the tubes together in a sharp line makes possible to compare the sealed tubes from the side even more accurat than open tubes viewed from above. The sealed tubes are gradua changed by solution of even the most resistant glass, but this cha has been reduced by increasing the volume-surface ratio as well the buffer and NaCl concentration. The selection of the brillia sulfophthalein indicators, brought to our notice by the work of Lu and Clark, greatly facilitates the determinations. We found that salt error of all of these indicators so far calibrated is the same; at le no distinction could be made within the range that we had to use.

Although we made the salt-content of the buffer mixtures the sa as sea-water, it is sometimes convenient to apply a slight salt correcti for the slight variation in the salinity of ocean-water rather th carry a separate set of tubes for each salinity. But the magnitude this correction seldom exceeds one-sixth of the correction that had to applied to Sörensen's buffer mixtures. In our final set of tubes, slight compromise was made in order to satisfy all of the principles s forth at once. Borax is the best buffer and is not very soluble; i solubility is reduced by the presence of salt. Since it was desired have as high a concentration of borax as possible, the salinity of tl buffer mixtures was made to equal that of sea-water of about tl lowest salinity ordinarily met with, and the labels of the tubes we changed so as to correct for salt error of sea-water of the mean salinit that is to be encountered in an investigation, and directions were give for correcting for variation in salinity. Unless otherwise specified tl tubes are labeled for a salinity of 31.25 grams per kilogram and wit these tubes salinity variation within the ordinary range of the large oceans causes slight errors that may be allowed for.

The buffer mixtures were made of different proportions of two stoc solutions. In making a set of tubes, the indicator was selected an added to portions of the two stock solutions in two automatic burette

FIG. 12



From J. F. McClendon's article in Publication 251, Carnegie Institution of Washington

in the proportion of 10 mg. per liter. The burettes were protected with soda-lime tubes and the condensation water was mixed up by shaking the burette occasionally. For this purpose a burette supported by the stock bottle is most convenient, since connections are not damaged in shaking. This burette is sometimes known as Squibb's automatic.

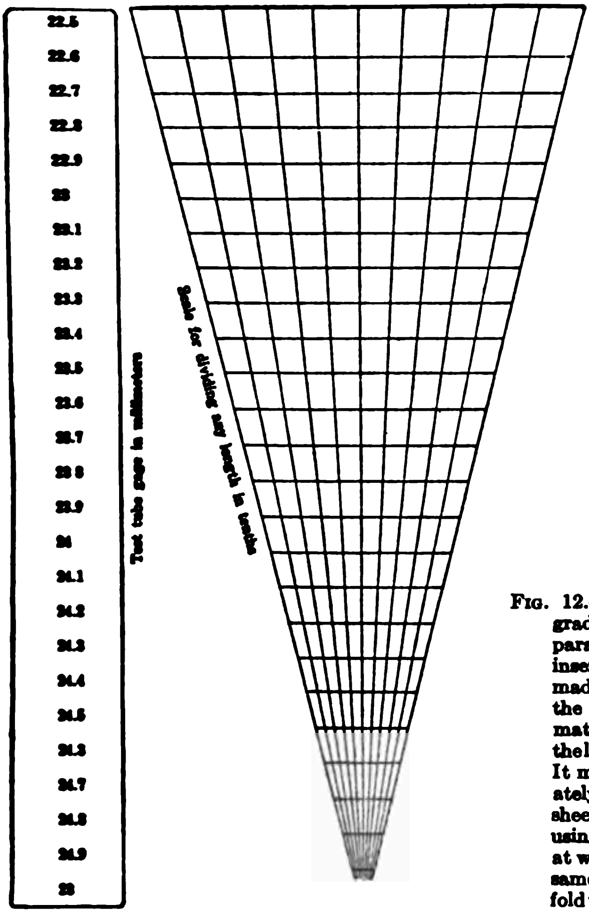


Fig. 12.—Test-tube gage and scale for graduating the special gasometric apparatus. The test-tube gage is to be inserted in the test-tube. Its slope is made very gradual in order to lessen the error produced by incorrect estimation of the position of the union of the lip and cylindrical part of the tube. It may be cut out and used immediately or after pasting it on a piece of sheet metal of the same shape. In using the other scale, find the position at which the width of the scale is the same as the length to be divided and fold the scale parallel to the heavy line.

A good glass as regards solubility is pyrex, but we have not become skillful enough to use glass of so high a melting-point. On account of its color Jena glass could not be used. The tubes used were nonsol test-tubes of 24 mm. bore. We obtained a very large number of these and divided them into grades in regard to bore, with the text-tube gage shown in figure 12. Those of the same bore and the most nearly cylindrical were selected for a set. The measurements were verified with a micrometer caliper, and no variation in any diameter greater than 0.2 mm. was allowed. Since there is danger of getting CO_2 into the tubes in sealing, they were first constricted in the middle to a very narrow neck, filled, and quickly sealed by applying the blast lamp to the center of this constriction, the part removed being saved for use with sea-water. The blast lamp and all other flames were in a separate room from the burettes. The seal left a point on the tube liable to be broken, but this was covered with a layer of melted chewing-gum followed by a cap of sealing-wax. In the following list of mixtures the first column gives the percentage of the acid solution having 0.3 m boric acid and 2.25 per cent NaCl, the second column the percentage of the alkaline solution of 0.075 m borax and 1.9 per cent NaCl, the third column gives the P_H, the fourth column the P_H of sea-water of S = 31.25 grams per kilo (Cl = 17.3 per kilo or 17.7 per liter) of the same color, and the fifth column the P_H of sea-water of S = 37.76 (Cl = 20.9 per kilo or 21.5 per liter) of the same color, the actual volume of acid solution used for a tube in the sixth column and of alkaline solution in the seventh column. Unless otherwise specified, the tubes are labeled according to the fourth column, and those of P_H 7.4 to 7.7 sterilized.

1	2	3	4	5	6	7	1	2	3	4	5	6	7
Per cent boric.	Per cent borax.	P_H Cl = 13.9 per liter all indicators.	P_H Cl = 17.7 per liter sulfophthaleins.	P_H Cl = 21.5 per liter sulfophthaleins.	C.c. boric.	C.c. borax.	Per cent boric.	Per cent borax.	P_H Cl = 13.9 per liter all indicators.	P_H Cl = 17.7 per liter sulfophthaleins.	P_H Cl = 21.5 per liter sulfophthaleins.	C.c. boric.	C.c. borax.
79.5	20.5	7.50	7.45	7.40	23.90	6.1	47	53	8.30	8.25	8.20	14.10	15.9
78	22	7.55	7.50	7.45	23.4	6.6	44.5	55.5	8.35	8.30	8.25	13.45	17.55
76	24	7.60	7.55	7.50	22.8	7.2	42	58	8.40	8.35	8.30	12.60	17.4
74	26	7.65	7.60	7.55	22.2	7.8	39.5	60.5	8.45	8.40	8.35	11.85	18.15
72	28	7.70	7.65	7.60	21.6	8.4	37	63	8.50	8.45	8.40	11.10	18.9
70	30	7.75	7.70	7.65	21.0	9.0	34.5	65.5	8.55	8.50	8.45	10.35	19.65
68	32	7.80	7.75	7.70	20.4	9.6	32	68	8.60	8.55	8.50	9.6	20.4
66	34	7.85	7.80	7.75	19.8	10.2	29	71	8.65	8.60	8.55	8.7	21.3
64	32	7.90	7.85	7.80	19.2	10.8	26	74	8.70	8.65	8.60	7.8	22.2
62	38	7.95	7.90	7.85	18.6	11.4	23	77	8.75	8.70	8.65	6.9	23.1
60	40	8.00	7.95	7.90	18	12	20	80	8.80	8.75	8.70	6.0	24.0
58	42	8.05	8.00	7.95	17.4	12.6	17	83	8.85	8.80	8.75	5.1	24.9
56	44	8.10	8.05	8.00	16.8	13.2	14	86	8.90	8.85	8.80	4.2	25.8
54	46	8.15	8.10	8.05	16.2	13.8	11	89	8.95	8.90	8.85	3.3	26.7
52	48	8.20	8.15	8.10	15.6	14.4	8	92	9.00	8.95	8.90	2.4	27.6
51	49	8.22	8.17	8.12	15.3	14.7	4.5	95.5	9.05	9.00	8.95	1.35	28.65
49.5	50.5	8.25	8.20	8.15	14.85	15.15	1	99	9.10	9.05	9.00	0.3	29.7

The first difficulty encountered was in obtaining a boric-acid solution with the same salt effect on the indicators as the borax solution. Starting from the conclusions of Palitzsch as to the salt value of boric acid, we finally developed the solution given. We have calibrated these tubes with sea-water about 50 times and in all parts of their range. Our tubes are labeled according to column 4 of the table, and include a set of o-cresolsulfophthalein tubes from P_H 7.45 to 8.30 and a set of thymolsulfophthalein tubes from P_H 7.9 to 9.05. The tubes deposit borax crystals when kept long at a low temperature and these crystals should be dissolved by warming the tubes before using them.

No special precautions are necessary in making the solutions. Reagent borax and boric acid needs no recrystallization, but commercial products must be recrystallized by dissolving in hot distilled water and filtering and cooling, then removing the mother liquor as much as possible and rinsing the crystals in ice-cold distilled water. The boric acid may be dried in a desiccator, but must not be heated above 70° to hasten the drying. Borax crystals effloresce in air on the surface, and powdery crystals must be discarded. It is usually sufficient to discard the surface layer of crystals from the bottle. The Na content of the borax may be titrated with 0.1 n HCl, using methyl red or dibrom-o-cresolsulfophthalein as indicator. It was found, however, that the eye was more capable of detecting efflorescence of the crystal before it was dissolved than by the use of methyl red afterwards. The other indicator is more sensitive and a flask containing the equivalent quantity of boric acid and NaCl may be used to get the end-point. The titration does not, however, distinguish between impurity and loss of water.

The borax solution may be prepared from boric acid and CO_2-free NaOH, but it takes more time to do this unless it is done at the same time that the solutions for determining the non-volatile buffer are prepared. Reagent NaOH contains more carbonate than is apparent on the surface of the sticks and must be purified. For this purpose, a saturated solution in distilled water is prepared (100 grams NaOH + 100 c.c. H_2O), and after thorough mixing it is sealed in a number of nonsol test-tubes by fusing the glass. These are placed upright in a thermos bottle (Dewar flask) or wrapped in cotton wool, in order to retard convection currents that stir up the precipitate. In 2 or 3 days the solution in the middle of each tube will be clear, the insoluble carbonate having settled, except for a little on the sides and the surface film. The only way to prevent a trace on the surface film is to completely fill the tubes, which can only be approximated. Owing to the high viscosity of the solution it can not be measured with delivery pipettes, and it is very convenient to have pipettes whose capacity is known (which can be determined by weighing when empty and when full of water and multiplying the difference by the density of water at that temperature). A tube is opened by cutting off the sealed end, the pipette tip is plunged to the center, and the sample quickly drawn. The meniscus is adjusted, the outside is wiped off, and the NaOH washed out of the pipette into a beaker with distilled water and titrated. If the opened test-tube is to be used again it must be quickly sealed with a lump of soft paraffin and inclosed in an air-tight container or CO_2-free compartment, preferably in a thermos bottle, for the disturbed carbonate to settle again; but it is advisable to use a tube only once. The solution of boric acid is prepared in a volumetric flask with distilled water freshly boiled in a metal container, but enough space is left to admit the NaOH, which is run in before the flask is filled to the mark. It is usually necessary to adjust the NaOH concentration by successive titrations and dilutions with freshly boiled distilled water before it can be measured with sufficient accuracy. All solutions should be sealed or provided with soda-lime tubes. The sealed tubes of saturated NaOH gradually become contaminated by the solution of glass. This process is slower in the borax solution, but it should be kept in the best resistance glass available.

Our boric acid was recrystallized several times in quartz beakers and the reagent borax was titrated, to make sure of the proper Na content. We standardized our HCl solution with reagent $NaHCO_3$ that had been heated to 270° to 300° (short of sintering) by Dr. F. B. Kingsbury, using methyl orange as indicator and a CO_2 solution for standard color of the end-point of the indicator. The weights used were standardized and volumetric apparatus certified and used at constant temperature. All of these precautions are probably not necessary, however, to duplicate our results. Owing to the high concentration of the buffers larger absolute errors are permissible than in Sörensen's buffer mixtures. The boric acid can be titrated in borax with CO_2-free NaOH, using phenolphthalein as indicator, provided large quantities of neutral glycerol are added to prevent hydrolysis. 1 m NaOH titrates 1 m H_3BO_3 (Treadwell-Hall). We consider this titration superfluous, provided the crystals do not appear to have lost water, and especially if the Na content is correct. It is probably less expensive to obtain pure borax than to titrate it.

A little care is necessary in comparing the color of the tubes. If parallel rays of light are passed through them they act as lenses, concentrating the light to a line and making comparison difficult. If ground (sand-blasted) glass is used to disperse the light, the granular appearance prevents the most exact estimation of the color. We found the only satisfactory background to be a piece of very thin opal glass placed close behind the tubes. The middle portions of the tubes then appear uniform. In order to increase the accuracy of the observations, we removed the lenses from a stereoscope, placed their inner edges together, and fastened them at such a distance (8.5 to 10 cm.) in front of the tubes that when one eye was closed and the other held about 6 inches from the lenses, the centers of the tubes were brought together in a sharp line. Two colorimeters of this nature were made and proved satisfactory. The only serious difficulty we had was in making the illumination symmetrical. This is easily done by allowing direct sunlight to fall on the opal glass of the colorimeter and adjusting a pair of gun-sights (that have been placed for the purpose on the top of the instrument) in line toward the sun, but the eye is made unfit for colorimetric work for some minutes unless a dark glass is used. Owing to the small part of the time during which we had sunlight, or even sufficient daylight, we tried mazda lights and found that we could compare o-cresolsulfophthalein (with some loss in accuracy) by means of a 250-watt stereopticon lamp, but this would not do at all for thymolsulfophthalein. The best artificial light we have tried is a 100-watt "daylite" mazda (blue bulb) with aluminium reflector, but we have great difficulty in making the light symmetrical, probably owing to irregularity in the thickness of the blue glass. This adjustment is made easier by a further dispersion of the light by means of a sand-blasted glass placed several inches behind the opal glass, but in every case it is necessary to verify a color-match by interchanging the tubes. The lamp was placed as close as possible to the tubes, but it might be supposed that the use of a larger lamp or thinner opal glass, thus enabling the lamp to be placed at a greater distance, would more nearly approximate the conditions with sunlight. We did not ascertain that the opal glass is colorless, although it appears pure white. The sand-blasted glass had a slight greenish hue noticeable on the edge, but we were unable to obtain colorless sand-blasted glass before the close of the experiments. The first attempt at using the colorimeter may lead to the supposition that the tubes may be matched more accurately by simply holding them before a piece of opal glass in the window, but this is due to failure of symmetrical lighting in the colorimeter. With a little experience it is possible to distinguish tubes in the colorimeter, which appear alike without it.

The indicators may be used in aqueous solution, since dilution of the sea-water has little effect on the P_H. In our experiments, however, it was necessary to use very concentrated solutions of the indicators, because we used the same sample of sea-water for the hydrogen electrode, the colorimeter, and the analysis of total CO_2, and dilution has an enormous effect on the CO_2 content at constant CO_2 tension. We made up 0.1 per cent solutions of the indicators in ethyl alcohol redistilled over sodium. The o-cresolsulfophthalein dissolved on heating, but the thymolsulfophthalein had to be assisted by a little CO_2-free NaOH. Enough NaOH to bring the color of the indicator to the middle of its range makes it more sensitive, but unless it is protected from CO_2 it will absorb quantities of this gas and become correspondingly insensitive. If NaOH is used to assist solution, it is safer to add an equivalent of HCl, as the small amount of this salt formed will not be appreciable when mixed with sea-water. Since we used only 0.1 c.c. of 0.1 per cent alcoholic solution of the indicator for 10 c.c. of sea-water, we do not believe the alcohol influenced the indicator method appreciably, but we suspect that both alcohol and indicator tended to depolarize the hydrogen electrode and increase the time necessary for equilibrium.

It is very difficult to completely prevent the concentration of the indicator solutions by evaporation while in actual use, and we did not attempt to do it. We kept the solutions in small Erlenmeyer flasks, each having a pipette, graduated in hundredths of a cubic centimeter, passed through the stopper into the solution. Perhaps the most convenient way of correcting the concentration of these solutions is to make up one tube of distilled water containing the same concentration of the indicator as the standard buffer mixtures, at the time the standard tubes are made. There is enough CO_2 in distilled water to turn these indicators to their maximum yellow color, yet not enough to carry them near their secondary color range (red in strong acid). The yellow color is constant over a very wide P_H range. It is then only necessary to determine the quantity of indicator solution necessary to color distilled water to the same degree of yellow as the sealed distilled-water tube, in order to have the amount necessary to put in the same volume of sea-water in determining the P_H. Even without this special tube, it is possible to determine colorimetrically (by repeated trials) the amount of indicator to add to sea-water; the color of each standard tube is the resultant of two colors (red and yellow or blue and yellow). It is impossible to match it with these colors in any other proportion, no matter how the concentration is changed. If the proper concentration of indicator is unknown, it may be found by repeated trials to match the color, using sea-water of any P_H within the range of the tubes. It was this consideration which led us to discard phenolphthalein and a-naphtholphthalein, since each showed only one color.

NEW APPARATUS FOR ELECTROMETRIC DETERMINATION OF HYDROGEN-ION CONCENTRATION AND FOR GAS ANALYSIS.

TECHNIQUE OF THE EXPERIMENTS.

The hydrogen electrodes were each made of a gold disk (later covered with a thin layer of palladium black) welded to a platinum wire sealed through the glass. There seems to be only one way to do this. A bit of gold is wound or folded over the end of the platinum wire and held in the flame until it just melts; then it is instantly removed. The congealed gold drop is then hammered on to the edge of the gold disk. The electrode is cleaned by filling the glass parts with a saturated solution of potassium bichromate in concentrated sulphuric acid (cleaning fluid) and rinsed thoroughly.

The palladizing solution is made by dissolving about 1 per cent of palladium chloride in distilled water and filtering. No reducing agent, such as basic lead acetate, is necessary. In fact, our solutions were weakened by spontaneous reduction, becoming dark with colloidal palladium; but this finally settled out, by further reduction, and the necessity of refiltering in order to watch the palladization was avoided.

The apparatus was filled with the palladium solution, a platinum wire was inserted for an anode, and a current of 4 volts passed until the gold became black. When necessary to repalladize, the apparatus was left full of nitric acid over night, which completely removed the palladium; it was then rinsed and the process was repeated. Palladium black does not seem to have the effective life of platinum black, but it was necessary to use it, as it can be removed without destroying the glass parts or the use of aqua regia. There is no necessity of electrolyzing H_2SO_4 in a hydrogen electrode, since any free chlorine is removed with the anode when it is taken out unless some aqua regia has been formed in it. If the palladium black is deposited too rapidly it will not stick.

We do not understand why a freshly palladized electrode behaves in a more reliable manner than one which has soaked in distilled water for many days. The electrode is thoroughly charged with hydrogen when palladized, but much of this is probably lost during the rinsing. Nitric and, to a smaller extent, other mineral acids attack palladium black and oxidizing agents probably affect it; hence we never cleaned palladized electrodes with cleaning fluid. It seems probable that the alcohol and indicator were slowly reduced by hydrogen at the surface of the palladium black, although we did not detect any decrease in the indicator concentration. It is probable that most of the alcohol had been removed from the solution by the current of gas before our final readings were taken. Such suppositions as to the effect of alcohol were based on variations of the potential of the electrode, and it is well known that such occur whenever free oxygen or oxyhemoglobin, that are reduced at the electrode, are present. The phenomenon is probably general, the ease with which the substance is reduced probably determining merely the rate at which reduction occurs. Even with a small amount of free oxygen in the solution, we found it possible to obtain the correct result by tilting the apparatus so that the platinized or palladized disk communicated with the main mass of fluid by only a thin film.

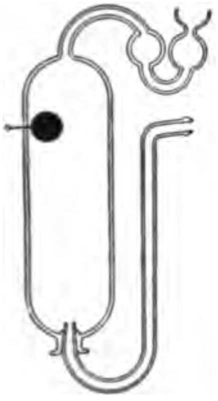
The oxygen is apparently used up in the immediate vicinity of the electrode in a relatively short time and the proximity to a large store of hydrogen hastens the resaturation of the electrode. But because of our ignorance of some of the factors involved, we did not wish to rely on any such assumptions in this set of experiments, and consequently we made about half of the determinations soon after immersion and half soon after removal of the electrode by tilting, and took the mean value. In some cases it was necessary to apply corrections for pressure of CO_2 and H_2 when they were not exactly the same in the gas and water phases, but these differences never exceeded 1 per cent of the partial pressure of the gas. We were very much impressed with the slowness with which equilibrium is established between the solution and the gas above it.

An experiment designed to illustrate this was made as follo 5 c.c. of sea-water were placed in the hydrogen electrode and a stre of pure hydrogen of about 100 c.c. per minute passed through About 15 minutes were required for saturation of the water wi hydrogen (so that the P_H was the same, 8.2, as with stagnant hydroge but with the electrode raised out of the water), and this time and minutes more were counted out of the experiment. The water no contained 0.2 c.c. of CO_2 in the form of carbonates and bicarbonate calculated from the P_H of 8.3. In 10 minutes, with the passage 1,000 c.c. of H_2 in small bubbles, the P_H rose to 8.4, indicating tha 0.007 c.c. of CO_2 had been removed. The rate of removal constantl decreased in an asymptotic curve, and at the end of 2 hours a P_H of was reached, indicating that a total of 0.05 c.c. of CO_2 had bee removed. During the next 10 minutes the P_H rose to 9.02, indicatin the further loss of 0.0014 c.c. of CO_2. At the end of 270 minutes the P_H had reached 9.18 and was rising at the rate of 0.01 in 10 minutes, indicating a total loss of 0.0616 c.c. and a final rate of 0.0007 c.c. per 10 minutes.

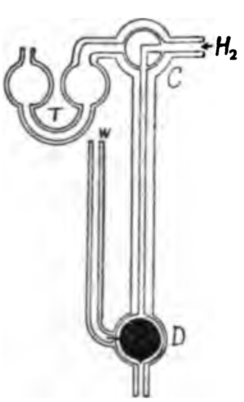
There was still enough CO_2 in the sea-water to convert most of the excess base into normal carbonate. This shows the hopelessness of reaching equilibrium at very low CO_2 tensions, and we seldom attempted anything lower than 0.0002 atmosphere, and then made tests to see that equilibrium was attained within the limit of error of measurement. The buffer mixtures were intended to be CO_2-free, and similar experiments on them showed that if traces of CO_2 were in them they were not removed by hours of bubbling hydrogen through them.

We know of no attempts to determine the asymptote or limit that is approached in the above experiment. If it is an NaOH solution that is approached, we should be able to remove any traces of CO_2 from the buffer mixture (theoretically with ideal apparatus). The weak acid in the buffer should help displace CO_2. Perhaps the reason we could not reach a higher P_H by bubbling H_2 through the buffer mixture was that the traces of CO_2 were not sufficient to measurably change the P_H. The electrodes for comparing the colors of the buffer mixtures and sea-water of the same P_H were made of the same bore as the sealed tubes and so that they could be placed in the colorimeter after determining the P_H electrometrically. The essential form of these is shown in figure 13. There are no ground joints or rubber connections exposed to the air. The hydrogen is allowed to escape through the trap at the top, which is filled with some of the same solution as in the electrode. Electrolytic connection with the calomel electrode is made through the ground joint at the bottom submerged in an intermediate vessel of saturated KCl solution.

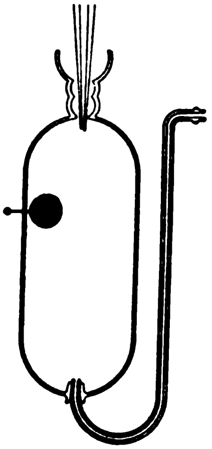
Some of the electrometric titrations were made with the dipping electrode shown in figure 14, which is rinsed with the solution by allowing the solution to rise in it by gravity and blowing it out with hydrogen. It is most convenient to make the vessel for holding the sea-water with a ground joint at the bottom for electrolytic connection. Select a glass-stoppered bottle, whose stopper does not leak, and cut off the bottom. This is done by cutting a short groove with a file or glass knife, and holding a heated rod just beyond each end of the cut until a crack is started. One heated rod is moved over the line chosen for the cut, at such a rate that the crack follows the rod. If metal rods or large nails can not be obtained, pieces of glass heated to a red heat on the end will do. After removal of the bottom the bottle is inverted and immersed to the neck in the KCl vessel. In making the titration, the sea-water is pipetted into the bottle and covered with a paraffined cardboard with holes for the electrode and burette tip.



13



14



15

FIG. 13.—Hydrogen electrode that can be placed in the colorimeter for calibrating the colorimetric method of determining the P_H, being of the same bore as the sealed colorimetric tubes. The ground joint at the bottom should not be greased, as it is for the electrolytic connection with the calomel electrode through the interposition of a KCl bath. The black circle is a palladised or platinised gold disk welded to a platinum wire fused through the glass. The long narrow tube is for the introduction of a continuous stream of hydrogen which escapes at the top through a trap which prevents the backward diffusion of air.

FIG. 14.—Dipping hydrogen electrode for titrations. Insert a drop of Hg and copper wire in *W*. The bulb *D* is immersed in the fluid in an inverted glass-stoppered bottle with the bottom cut off. The ground joint at the stopper serves for electrolytic connection with the KCl bath. The cock *C* is turned so that the bulb *D* fills by gravity with the fluid, which drives out the hydrogen at the trap *T*. Hydrogen is allowed to bubble through again and the fluid allowed to rise until it just touches the palladised gold disk shown in black, when the reading is taken. (From Jour. Biol. Chem., 1916, xxviii, 135.)

FIG. 15.—Hydrogen electrode for CO_2-free electrometric titrations. The principles of construction are the same as in figure 13, except that the trap at the top is made to admit a burette tip, and can be sealed with a drop of distilled water after the burette tip has been inserted so as to leave only a very narrow opening.

A more convenient form of titration electrode is shown in figure 15. It has the advantage that the air is excluded from the whole solution by lowering the burette tip far enough into the hole in the top to seal with a water seal. Hydrogen is passed through for about 15 minutes before commencing the titration and is continued all through the titration, escaping through the water seal, which should be replenished with a drop of distilled water in case it is blown out by the hydrogen.

The most difficult problem was to bring the sea-water in the hydrogen electrode to a known CO_2 tension. Various forms of apparatus for treating the sea-water with gas mixtures were tried, but it was found that a very long time was required to reach equilibrium. We finally devised a simple apparatus in which equilibrium could be reached in 30 minutes or less (although we took more time to make sure of it after the first time tests were made). This tonometer electrode (fig. 16) consists of a tube 450 mm. long and 7 mm.

16

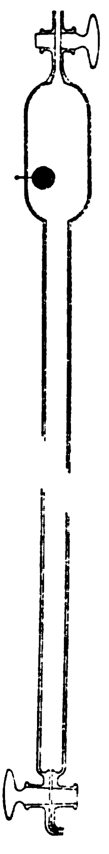


FIG. 16.—Tonometer electrode for determining the P_H of sea-water at a predetermined CO_2 tension. A mixture of CO_2 and H_2 is bubbled through the sea-water in a narrow tube; then the stopcocks are closed and the electrode inverted so that the sea-water flows into the wide portion, which is of the same bore as the colorimeter tubes, so that the hydrogen electrode determination of the P_H may be compared with that by the colorimetric method. The stopcock on the wide end of the electrode is not greased, as it serves for electrolytic connection with the KCl bath.

17

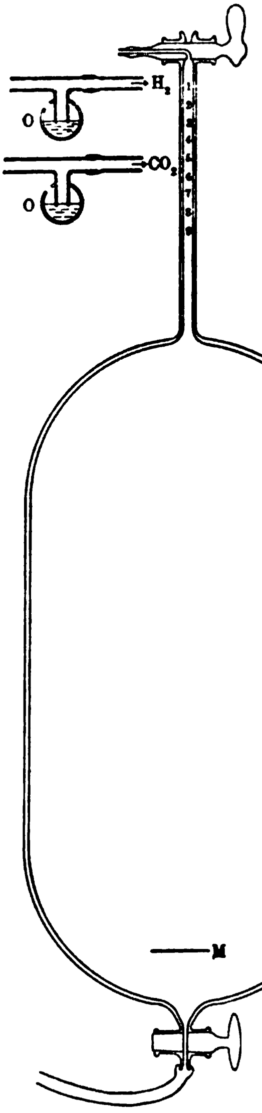


FIG. 17.—Gasometer for mixing CO_2 in ten-thousandth parts with H_2 (atmospheric pressure being insured by the traps, *OO*). The apparatus holds 25 pounds of Hg and hence must be bound with iron wire run through boiling sealing-wax while being wound on diagonally. The narrow tube is graduated in tenths or hundredths of a cubic centimeter. The apparatus holds 1 liter of gas when the mercury meniscus is at *M*. The proportions are not quite correct in this figure. It is best to have the apparatus narrower and so that *M* may be marked on the cylindrical part; or (as we accomplished in one apparatus) *M* may be at the lower stopcock. The lower stopcock is connected by means of a piece of pressure rubber tubing to a leveling bulb held up by a rope passing through pulley attached to the ceiling.

bore with a stopcock at one end and the other end fused to a short tube of exactly 24 mm. bore, containing the palladized gold disk and closed with a minute ungreased stopcock. About 11 to 12 c.c. of sea-water containing the indicator were sucked up into it and it was clamped with the small tube downward (which then contained a column 30 cm. high of sea-water). The mixture of CO_2 and H_2 was passed in at the bottom in small bubbles, and the flow regulated so that the sea-water did not rise into the large tube. The gas mixture escaped at the top through a trap connected to the stopcock by a short rubber tube, to prevent the backward diffusion of air. The gas pressure of the bubbles varied from the barometer reading to the barometer + 300 mm. of water, the average being barometer + 150 mm. of water or about barometer + 11.5 mm. of mercury.

We tested equilibrium by first starting with sea-water of a known lower CO_2 tension than the gas mixture, and making a second determination on sea-water of a known higher CO_2 tension than the gas mixture. If the final P_H was the same in both cases we concluded that equilibrium had been sufficiently approximated, but in all later experiments we about doubled this time.

In making the electrometric reading the electrode was simply inverted so that the sea-water ran down into the large tube, the ungreased stopcock immersed in the KCl bath, and a platinum-tipped wire hooked into the platinum loop to connect to the potentiometer. If the reading was taken with the disk out of the water, the H_2 and CO_2 partial pressures were the same as in the original gas mixture, but if the disk was immersed, 11.5 was added to the H_2 pressure and $p\left(\frac{11.5}{\text{bar}}\right)$ was added to the CO_2 pressure in making the calculations. The pressures were always corrected for vapor tension of water before the final calculations.

The gas mixture was made in the apparatus shown in figure 17, holding about 25 pounds of mercury. This consisted of a liter separatory funnel with a narrow tube and 3-way cock attached at the top, and wrapped with wire passed through hot sealing-wax to increase its strength. By graduating the narrow tube in tenths of a cubic centimeter and making a mark, *M*, near the lower end of the separatory funnel to denote a total volume of a liter, it was possible to measure the CO_2 in parts per 10,000. The CO_2 was washed with $NaHCO_3$ solution and the H_2 with $HgCl_2$ solution and again in H_2O in wash bottles shaped like pyknometers, and passed through traps (*O*, fig. 17) to insure atmospheric pressure. The apparatus was filled with mercury by raising the leveling bulb by means of a rope passing through a pulley in the ceiling. The CO_2 apparatus was attached to the 3-way cock and the air was washed out of the connections. The 3-way cock was now turned as in figure 17, so that CO_2 entered the narrow tube when the leveling bulb was lowered and the lower stopcock of the sepa funnel was opened cautiously. When the required amount of C admitted, the lower cock was closed, the 3-way cock reversed, and apparatus substituted. The CO_2 was washed out of the connectio the 3-way stopcock turned as in figure 17 and H_2 admitted by o the lower stopcock. The operator kept his hand on this stopcoc the filling was complete, because the H_2 came faster at first tha and there was danger of the mercury leaving the separatory faster than the H_2 was supplied, in which case water would be in from the trap *O*. It was necessary to allow a little H_2 to continuously through the trap *O*, as a matter of safety. Wh

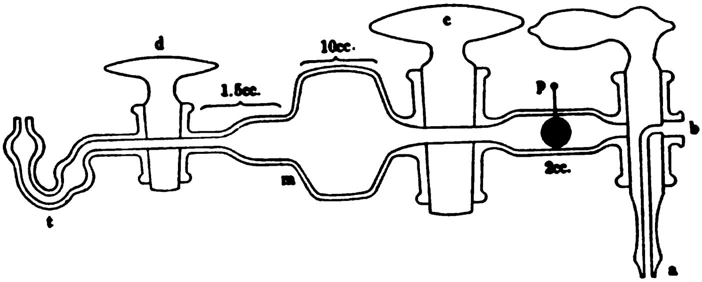


Fig. 18.—Hydrogen electrode for determining the P_H of water taken directly from the sea. A drop of water is placed in the trap *t*, to seal it, and the electrode is filled with pure hydrogen. The hole in the 3-way cock is filled with sea-water by sucking on a rubber tube attached to *b* while *a* is immersed in the water; then this cock is reversed and the suction applied at *t* until the sea-water rises to the mark *m*, when all cocks are closed. The 1.5 c.c. of hydrogen remaining in the electrode is shaken with the 10 c.c. of sea-water in the large compartment until it has the same CO_2 tension as the sea-water, when it is passed into the small compartment by opening the large cock *c* (which must be 4 mm. bore and not greased) and tapping the electrode. *D* is opened a moment before *c* is closed to reestablish atmospheric pressure and the electrode is shaken again before taking the reading. Electrolytic connection is made through the ungreased cock *c*. A wire is hooked in *p* to connect with the potentiometer.

mercury meniscus reached the lower mark, the cocks were close the apparatus shaken, the remaining mercury stirring and mixi gas. The tonometer electrode was now attached, the leveling was raised, and the gas mixture was passed through the tall colu sea-water in fine bubbles, which was accomplished only after a c adjustment of all the stopcocks.

For the determination of the P_H of sea-water taken directly fro sea, the electrode shown in figure 18 was used. It was first fille H_2 and then sea-water was admitted through *a* to the mark *m*, washing out the air at *b*. The H_2 was shaken with 10 c.c. of sea- in the large compartment; then by tapping the apparatus it was c to enter the smaller compartment and again shaken. In this wa loss of CO_2 by the second portion of sea-water was minimized.

ungreased stopcock was immersed in the KCl and the reading was taken in the usual manner.

The calomel electrodes were made from mercury redistilled in Hulett's still (and plated on platinum so as to be portable), KCl was recrystallized many times in quartz beakers, and calomel was made by the electrolytic method of Lipscomb and Hulett. The 0.1 n KCl calomel electrodes were made at 20° and hence have a very slight error at 10° and 30° due to volume change of the KCl solution in them. The KCl was weighed with standard weights and the weighings reduced to vacuo. A saturated KCl calomel electrode was often used as an intermediate on account of its lower resistance, but each time was compared against the 0.1 n electrode. The saturated electrode often varied about 1 millivolt, although the readings were never taken in less than 3 hours after the room was brought to constant temperature, after a maximum fluctuation of about 2 degrees.

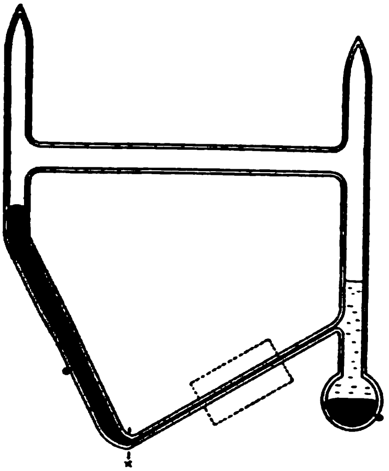


FIG. 19.—Capillary electrometer of increased sensitivity. The large bulb is inclined toward the observer by bending the neck while the glass is soft, so that the electrometer will lie flat on the stage of a microscope. It is attached in this position by means of rubber bands and the microscope is tilted back until the tube is horisontal. Wires are hooked in the little rings, which are platinum loops fused through the glass. The dotted rectangle represents a cover-glass mounted on the capillary with Canada balsam. The capillary must be the smallest obtainable and of thin enough walls to admit of an 8 mm. objective and x 20 ocular in the microscope. A few specks of dust in the ocular will serve to locate the sero-point, and a micrometer scale is not necessary.

Two special potentiometers made by Leeds and Northrup were used. One was standardized by the Bureau of Standards and the other compared with it. One of these is now at Tortugas and the other in Minneapolis. The special features are the time-saving arrangement and key which automatically short-circuits the capillary electrometer when not in use.

We had difficulties with the ordinary forms of capillary electrometers, but found the slight modification shown in figure 19 satisfactory. It was always necessary to make sure the electrometer was not polarized before taking a reading. The sensitivity was made sufficient by using the smallest capillary tubing obtainable and a microscope of very high power, especially in the ocular. The electrometer was attached to the stage of a tilted-back microscope with rubber bands. If polarized, it is tilted sidewise until a drop of mercury

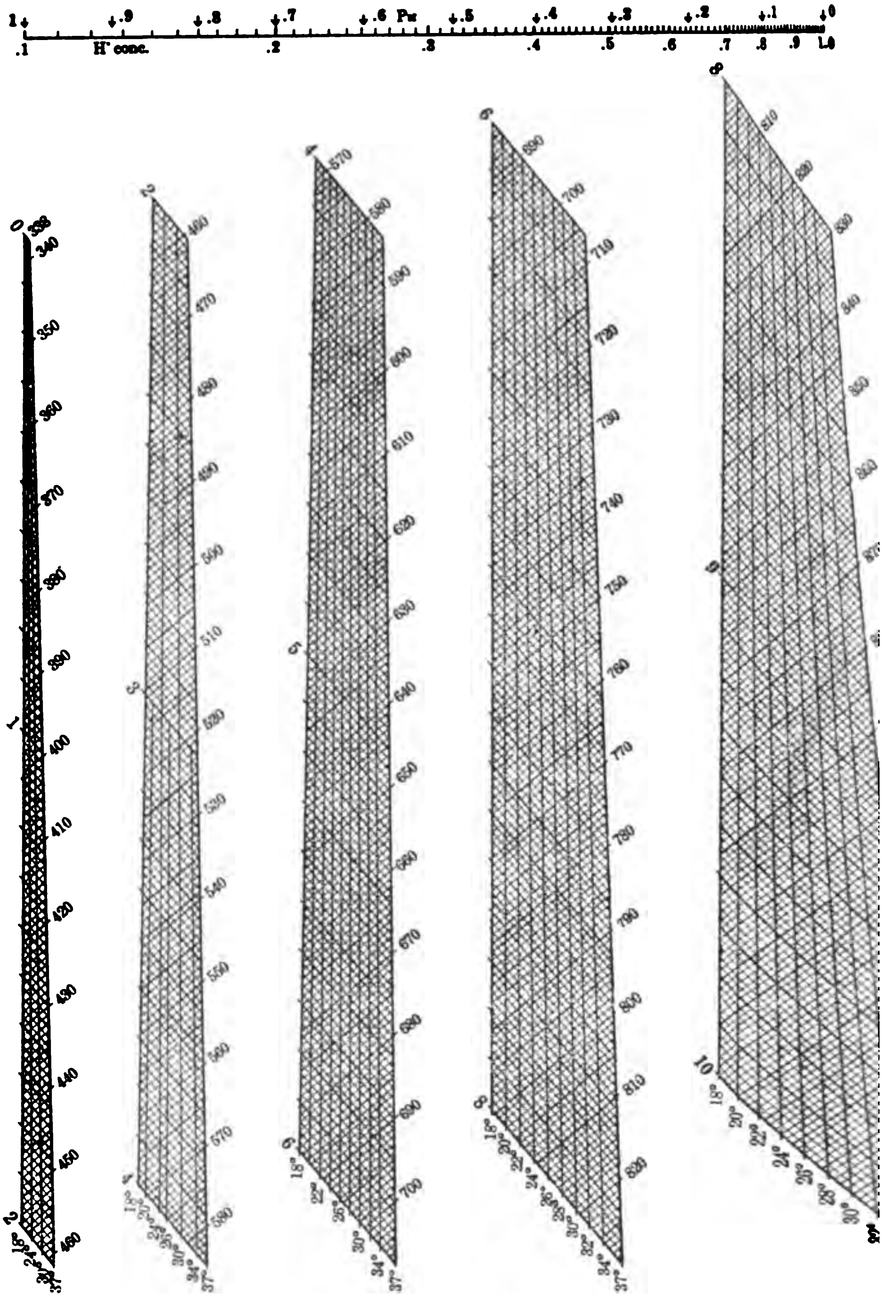


FIG. 20.—Table for conversion of millivolt readings into P_H when using a hydrogen el connected with a 0.1 n KCl calomel electrode. A correction for the concentration of hy gas must be added to millivolt reading before using the table. The large figures from denote P_H, the figures from 338 to 950 denote millivolts, and the temperature li designated at the bottom. The double scale at the top is for conversion of the fractio of P_H into the digits used in the decimal system, the decimal-point being determined integral part of the P_H. We see no advantage in the decimal system over the loga (P_H) system of expressing H-ion concentration, but have added this scale for compa results expressed in the two systems. In the upper double scale the arrow-heads tenths of a P_H and the lower scale the digits of the decimal system. Thus $P_H = 8.7$ is ab same quantity as H' conc. $= 0.2 \times 10^{-8}$. P_H 7 was misplaced.

falls out of the capillary. A cover-glass is mounted on the capillary with balsam.

Before using the conversion table for converting millivolts into P_H a slight correction given in the following table was added to the millivolt reading, on account of the rarefaction of the hydrogen.

Barometer.	10°					20°					30°				
	Per cent of hydrogen.					Per cent of hydrogen.					Per cent of hydrogen.				
	100	99	98	95	90	100	99	98	95	90	100	99	98	95	90
780	0.1	0.2	0.3	0.6	1.3	0.9	1.0	1.2	1.6	2.3	1.7	1.8	1.9	2.3	3.0
770	0.3	0.4	0.5	0.8	1.5	1.1	1.2	1.4	1.8	2.5	1.9	2.0	2.1	2.5	3.2
760	0.5	0.6	0.7	1.0	1.7	1.3	1.4	1.6	2.0	2.7	2.1	2.2	2.3	2.7	3.4
750	0.7	0.8	0.9	1.2	1.9	1.5	1.6	1.8	2.2	2.9	2.3	2.4	2.5	2.9	3.6
740	0.9	1.0	1.1	1.4	2.1	1.7	1.8	2.0	2.4	3.1	2.5	2.6	2.7	3.1	3.8
730	1.1	1.2	1.3	1.6	2.3	1.9	2.0	2.2	2.6	3.3	2.7	2.8	2.9	3.3	4.0

The above table is calculated on the hypothesis that the potential of the hydrogen electrode in a normal solution of H ions depends on the concentration and not solely on the pressure of the hydrogen. Hence the value of the hydrogen electrode against the 0.1 n KCl calomel electrode is not 337 millivolts as used in case no correction for hydrogen pressure has been applied, but a slightly higher value. The average of the determinations listed by Clark and Lubs for temperatures up to

Table for converting millivolts into P_H *at 10°.*

P_H	+0.0	+0.1	+0.2	+0.3	+0.4	+0.5	+0.6	+0.7	+0.8	+0.9
3	506.45	512.1	517.7	523.3	528.9	534.5	540.1	545.7	551.3	556.9
4	562.6	568.2	573.8	579.4	585.0	590.6	596.2	601.8	607.4	613.0
5	618.75	624.4	630.0	635.6	641.2	646.8	652.4	658.0	663.6	669.2
6	674.90	680.5	686.1	691.7	697.3	702.9	708.5	714.1	719.7	725.3
7	731.05	736.7	742.3	747.9	753.5	759.1	764.7	770.3	775.9	781.5
8	787.20	792.8	798.4	804.0	809.6	815.2	820.8	826.4	832.0	837.6
9	843.35	849.0	854.6	860.2	865.8	871.4	877.0	882.6	888.2	893.8

Table for converting millivolts into P_H *at 30°.*

P_H	+0.0	+0.1	+0.2	+0.3	+0.4	+0.5	+0.6	+0.7	+0.8	+0.9
3	518.4	524.4	530.4	536.4	542.4	548.4	554.4	560.4	566.4	575.4
4	578.5	584.5	590.5	596.5	602.5	608.5	614.5	620.5	626.5	632.5
5	638.6	644.6	650.6	656.6	662.6	668.6	674.6	680.6	686.6	692.6
6	698.7	704.7	710.7	716.7	722.7	728.7	734.7	740.7	746.7	752.7
7	758.8	763.8	770.8	776.8	782.8	788.8	794.8	800.8	806.8	812.8
8	818.9	824.9	830.9	836.9	842.9	848.9	854.9	860.9	866.9	872.9
9	879	885	891	897	903	909	915	921	927	933

40° (which is the highest necessary in biological work) is 338.15, we have rounded off this value of 338 for all temperatures betwee and 40°.

After adding the correction from the above table, the milli reading was converted into P_H by the conversion table in figure which was cut into five sections to save space. The larger numb (1–10) denote P_H, the numbers 338 to 950 denote millivolts, and the vertical lines temperatures, as indicated below. The double horizontal scale above is for converting the fractional part of the P_H into the coefficient used in expressing the hydrogen-ion concentration, tenths of a P_H being denoted by the arrows above, and the coefficient by the finely divided scale below.

The table at 10°, near bottom of page 57, serves to extend the range, and the table at 30°, just below, serves as a guide to figure 20.

In choosing the size of the apparatus for the analysis of the total CO_2 in the sea-water, it was necessary to consider the fact that we had to bring the water sample to a known CO_2 tension before the analysis. We found it impossible to make more than a liter of the gas mixture correctly. We made gasometers on a larger scale, but they were unsatisfactory. It was also impossible to bring a large sample of sea-water to a known CO_2 tension with only 1 liter of gas mixture. We finally chose the micro-apparatus used by Van Slyke in determining the total CO_2 in blood-plasma. The large compartment of our apparatus held 50 c.c., but we would recommend its increase to 100 c.c. The form we used in the first part of the work was essentially like figure 21, but later we cut off the absorption pipette *P*, and absorbed the CO_2 by running NaOH solution directly into the gas burette *B*.

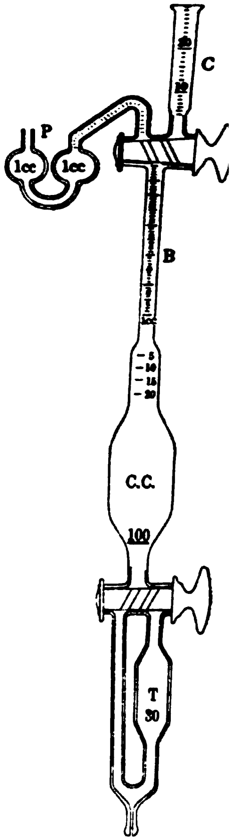


Fig. 21.—Van Slyke plasma CO_2 apparatus as used for the determination of the total CO_2, O_2, and N_2 of sea-water. The absorption pipette *P* may be omitted and a simple stopcock used on the top. A piece of small-bored suction rubber tubing, 80 cm. long, is attached to the lower end of the apparatus, and connected to a leveling-bulb with cylindrical sides that is held up by means of a swivel attachment to a screw passed through a nut held in a burette clamp attached to a tall rod support for the whole apparatus. The essential feature of the apparatus is the graduation of the gas burette *B* in hundredths of a cubic centimeter, so that the gas-volume can be accurately determined with the apparatus in the upright position and an upturned water meniscus below the gas. The graduations should go at least half-way around the gas burette to avoid parallax errors. It should be possible to estimate thousandths of a cubic centimeter by means of a lens.

The following description applies to the 50 c.c. apparatus without the absorption pipette. We would recommend the substitution of a simple 2-way stopcock for the one at the top.

The apparatus was clamped upright to a vertical iron rod rising 6 feet from the floor, and a horizontal rod was placed under the lower stopcock to prevent it slipping out of the clamp. The ordinary clamps used in chemistry were used, and the vertical rod supported in the middle by attaching it to the edge of a table with a staple, and at the bottom by inserting it into a hole in a board nailed to the floor. A leveling bulb with cylindrical sides was attached by means of soft-rubber suction tubing (1 mm. bore and 800 mm. long) to the lower end of the apparatus. The leveling bulb was attached by means of a swivel to a screw (of about 6 mm. pitch) and passing through a nut held in a burette clamp on the rod. Another burette clamp was attached to the rod about 800 mm. lower down, and the nut could be changed from one clamp to the other in a few seconds. The stopcocks were greased with a mixture made by dissolving pure gum-rubber (containing no sulphur) in boiling paraffin, and thinning with vaseline. This preparation was satisfactory at 10° and at 20°, but a more satisfactory preparation at 30° may be prepared from sapota-tree sap.

The apparatus was completely filled with mercury and exhausted once by closing the upper stopcock and lowering the leveling bulb, in order to practically free it from air. The bulb was raised again and the air-bubble forced out of the top. The mercury in the cup *C* was allowed to run down until it filled only the capillary neck. The leveling bulb was lowered below *B* and 10 c.c. of sea-water were allowed to run quickly from the tonometer electrode into the cup *C*, and then quickly into the burette *B*, care being taken that the capillary neck of *C* remained full of sea-water. 1 c.c. of 2 n HCl (*i. e.*, acid with about the same absorption coefficient for the elementary gases as the average sea-water) was introduced into *C* and allowed to run down into *B*. A little mercury was placed in *C* and some of it was allowed to run down into *B*, so that the hole in the stopcock was filled with it and closed. If the stopcock had two holes, as in the figure, the other hole and connecting tube had already been filled with mercury in filling the apparatus with mercury. This mercury effectively sealed the middle of the stopcock, and it seldom leaked, especially if more grease was smeared over the two ends of the stopcock. The stopcocks were usually greased again for each determination. Air getting in at first makes no theoretical difference, but leakage may ruin the determination by occurring at the last.

By lowering the leveling bulb, the mercury meniscus was brought to the 50 c.c. mark (100 c.c. in fig. 21). The lower cock was closed and the apparatus removed from the clamp and held with the left hand at the attachment of the rubber tube and the right hand at the upper tip of the cup *C*, and shaken violently laterally while in the vertical position, for 2 minutes exactly. In this way the sea-water was brought into approximate equilibrium with the gas pumped out of it. The apparatus was clamped again and the lower cock turned so that the sea-water passed into the trap *T*, the screw being adjusted so that the water meniscus was brought exactly to the upper entrance to the hole in the lower stopcock. One minute was allowed for the sea-water to drain down; then the lower stopcock was reversed and the leveling bulb raised so that the gas in the gas burette *B* was brought to atmospheric pressure. One minute was allowed for the moisture caught between the glass and the mercury to rise, and then the sea-water on top of the mercury was carefully measured and recorded. It should be about 0.05 c.c. The leveling bulb was then raised sufficiently to make the mercury meniscus rise half the height of the water column. The lower stopcock was closed and the leveling bulb brought back to the same level as the mercury in *B*.

The apparatus was now removed from the clamp and placed horizontally, with the trap *T* upwards. By little jerks the mercury was forced into *B*, thus displacing the gas and water into the wide part of the apparatus. It was shaken in this position 1 minute, and returned to the clamp, care being taken that no water worked around from *T* and rose into *B* when the lower cock was opened again. On openin this lower cock, the mercury meniscus should not rise or fall more tha a small fraction of a millimeter. The leveling bulb was now carefull adjusted with the screw so as to bring its meniscus on exactly the sam level as the mercury meniscus in the burette *B*. This is not at first a easy matter, but may be assisted by placing the leveling bulb directl behind the burette and placing 2 specks of dust on the mercury in i so that it is possible to bring the meniscus in *B* and the two specks dust in the leveling bulb in line.

A cylindrical bulb should be used, as a pear-shaped bulb is liable lead to error from refraction. The water meniscus is now careful read, so as to determine the total gas volume, and recorded. It i great advantage to have the graduations on *B* run at least half-w around the tube so as to avoid parallax errors, as an error of 0.001 means an error of 0.1 c.c. CO_2 per liter. We used a thermometer l for reading the meniscus. About 0.5 c.c. of half-normal CO_2-f NaOH was placed in *C* and allowed to run down into *B* a little a time while tapping the apparatus.

Complete absorption of CO_2 usually took place in about 5 minu at 20° or 10°, but at 30° the vaseline floated out of the stopcock gr and over the surface of the alkali, and it sometimes required 30 min for complete absorption. Half-normal NaOH has about the s absorption coefficient for the elementary gases as the average water, but it may be possible to use a stronger solution without ma gas-bubbles rise from the little sea-water above the mercury. We prefer the half-normal solution, however, because it is completely neutralized and washed out at the end of an experiment by the sea-water from the trap *T*, and it is only necessary to grease the stopcocks before another determination. In reading the gas-volume after absorption it is necessary to level the bulb, reckoning $\frac{1}{13}$ of the NaOH column as mercury.

The calculation of the results are a little complicated, but it was impossible to make a conversion table possessing great accuracy. We found the gas burette in one apparatus correct for the upturned meniscus, but it was therefore incorrect for measuring the water between the upturned and downturned menisci. A small correction in cubic millimeters given in the following table must be added to the volume of water above the mercury as measured by the scale on the gas burette *B*, depending on the total length of this scale in centimeters.

Length of 1 c.c.	Correction in c. mm.	Length of 1 c.c.	Correction in c. mm.	Length of 1 c.c.	Correction in c. mm.	Length of 1 c.c.	Correction in c. mm.	Length of 1 c.c.	Correction in c. mm.
5	24	11	11	16	8.4	21	6.7	26	5.6
6	17	12	103	17	8	22	6.4	27	5.4
7	15	13	9.7	18	7.6	23	6.2	28	5.3
8	13.5	14	9.2	19	7.3	24	6	29	5.1
9	12.5	15	8.7	20	7	25	5.8	30	5
10	11.7								

In order to make certain corrections, it is necessary to know the absorption coefficient of neutralized sea-water for CO_2. Since the half-normal HCl added to the sea-water has about the same absorption coefficient for atmospheric gases as neutralized sea-water, we assume that it does not seriously affect the absorption coefficient of the sea-water when 1 c.c. is added to 10 c.c. of the latter. Bohr's data were plotted for this purpose on the assumption that the absorption coefficient of neutralized sea-water for CO_2 is the same as that of NaCl solution of the same Cl content. The isotherms form characteristically curved lines, but in the small portion included in figure 22 the curvature was hardly perceptible and was later obliterated by the use of a straight-edge in inking-in the pencil drawing.

It is necessary to know capillary depression of the mercury in *B*, which is easily determined by opening all stopcocks and measuring the vertical distance between the mercury meniscus in *B* and the plane of the mercury surface in the leveling bulb. A mercurial barometer with glass scale is preferable, and the capillary depression must be subtracted from the reading.

In the following table for reducing the final gas-volume to 0°, 760 mm., and dryness, the temperature correction for the glass scale barometer is included. The moist gas-volume at designated temperature and pressure is multiplied by the correction in the table. Degrees and fractions of a degree may be estimated, because 1° makes about as much difference in the correction as 4 or 5 mm. pressure.

	10°				20°				30°			
	730	740	750	760	730	740	750	760	730	740	750	760
0	.9133	.9260	.9387	.9513	.8703	.8825	.8947	.9069	.8230	.8350	.8470	.8590
1	.9146	.9273	.9400	.9526	.8715	.8837	.8959	.9081	.8242	.8362	.8482	.8602
2	.9158	.9285	.9412	.9538	.8727	.8849	.8971	.9093	.8254	.8374	.8494	.8614
3	.9171	.9298	.9425	.9551	.8740	.8862	.8984	.9106	.8266	.8386	.8506	.8626
4	.9184	.9311	.9437	.9563	.8752	.8874	.8996	.9118	.8278	.8398	.8518	.8638
5	.9197	.9324	.9450	.9576	.8764	.8886	.9008	.9130	.8290	.8410	.8530	.8650
6	.9209	.9336	.9463	.9589	.8776	.8898	.9020	.9142	.8302	.8422	.8542	.8662
7	.9222	.9349	.9475	.9601	.8788	.8910	.9032	.9154	.8314	.8434	.8554	.8674
8	.9235	.9362	.9488	.9614	.8801	.8923	.9045	.9167	.8326	.8446	.8566	.8686
9	.9247	.9374	.9500	.9626	.8812	.8935	.9057	.9179	.8338	.8458	.8578	.8698
10	......	......	......	.9639	......	......	......	.9191	......	......	......	.8710

The mode of calculation is perhaps best shown by the following example: The analysis was made on 10 c.c. of sea-water of Cl = 19.375 per kilo, and the room was 20°, so the absorption coefficient was 0.765. The length of the graduated portion of the burette *B* was 9 cm., so the meniscus correction (Hg-H_2O and H_2O-gas) was 0.0125 c.c. and the capillary depression 3 mm. The barometer minus the capillary depression was 725 mm. The water above the mercury meniscus was $0.0565+0.0125=0.069$ c.c. The total gas (0.54) minus the gas after absorption of CO_2 (0.179) equals the CO_2 (0.361 c.c.) as measured directly; but the 0.069 c.c. of water was in equilibrium with a gas, a large fraction (0.669) of which was CO_2; hence the CO_2 in that water was $0.069\times0.669\times0.765=0.353$. In other words, the CO_2 in the water = its volume × the CO_2 fraction + the absorption coefficient.

Practically all of this CO_2 was pumped out and then reabsorbed; hence the amount originally pumped out was $0.361+0.0353=0.3963$ c.c., and this amount expanded to 39 c.c. was in equilibrium with 11 c.c. of water at the end of the first shaking. Hence this 11 c.c. finally trapped off in *T* contained $11\times\frac{0.3963}{39}\times0.765=0.0855$ c.c. of CO_2. This added to the 0.3963 c.c. brings the total up to 0.4818 c.c., and this $\times0.8644=0.4165$ c.c. per 10 c.c. or 41.65 c.c. per liter at 0° and 760 mm. This correction of 0.8644 goes outside the table, but the table is probably extensive enough for most determinations, especially at a lower altitude, and the logarithm of the corrections for a more extended range may be found in the Chemiker Kalender or Landolt-Börnstein.

The apparatus was tested by making an artificial sea-water of neutral salts, distilled water that had just been boiled 15 minutes in a quartz vessel and cooled with CO_2-free air passing through it, and some of the Na_2CO_3 used in standardizing the HCl, and weighed with standardized weights. The total CO_2 as calculated and found in this solution differed by about 0.5 per cent.

Dole used a simpler (titration) method for determining the CO_2 in sea-water, but we have not yet had time to compare it with our

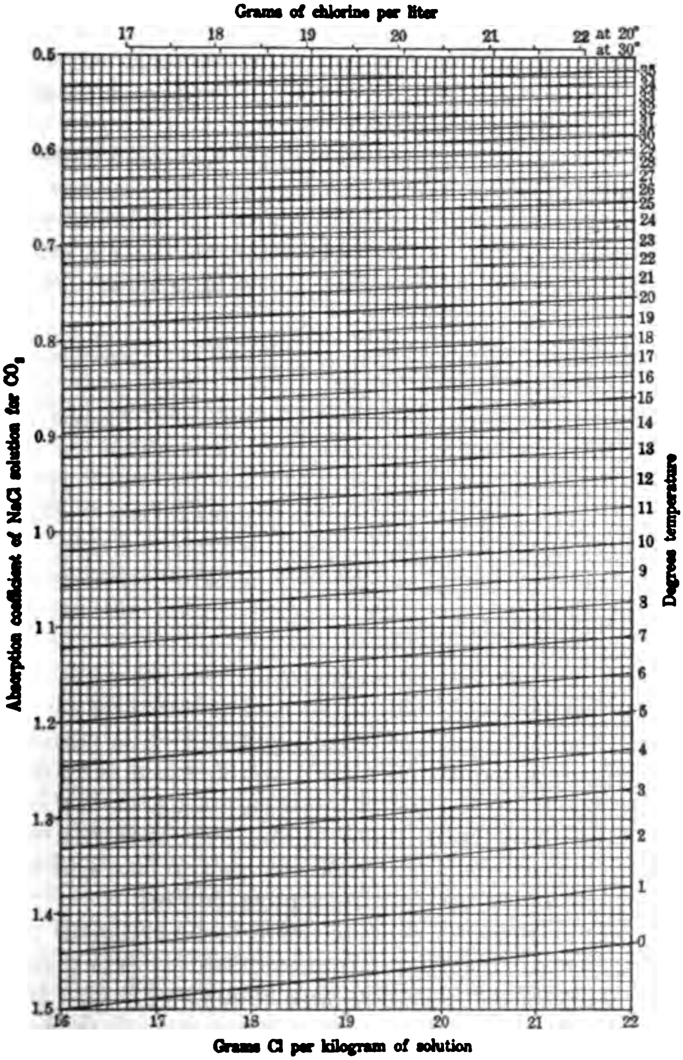


FIG. 22.—Conversion table for finding the absorption coefficient of acidified sea-water for CO_2. This table was taken from the center of a graph plotted from Bohr's data on the absorption coefficients of distilled water and solutions of NaCl for CO_2 on the assumption that the absorption coefficient of sea-water and salt solution of the same Cl content are the same, provided the sea-water does not contain alkali to combine with the CO_2. The error that may be the result of this assumption is necessarily minute and insignificant compared with errors that might arise from slightly incorrect thermometers. Each diagonal is for a different temperature. The grams of Cl per kilogram of sea-water are indicated below and grams of Cl per liter of sea-water at 20° and again at 30° is indicated above.

results. We could not use it in these experiments on account of the large sample required.

The oxygen in the sea-water may be determined on the same sample after the CO_2, by absorbing it with an alkaline solution of pyrogallol, introduced in the same manner as the NaOH solution, and the nitrogen + other inert gases may be determined by the residue. The whole calculation must be repeated for these gases, using their respective absorption coefficients, as given in the following table, calculated from Fox's data, for sea-water of Cl = 20. Fox does not state whether this is Cl per liter or per kilogram.

One of the most important requirements in such investigations as are described in this paper is accurate temperature control. All of the determinations in Minneapolis were made in a laboratory that was converted into a constant-temperature room by cutting off the steam heat and regulating the temperature by means of a nichrome wire stretched through the air in front of an electric fan and carrying 1,600 watts. The relay controlling the heating current was burned by arching because it was too small and we did not obtain a large one in time. But we found it possible to control the temperature to 0.1° by personal attention to a sensitive thermometer (graduated in tenths of a degree) placed in front of a one-sixth horse-power electric fan, and the heating current was controlled by means of a push-button.

Degrees.	Absorption coefficient of sea-water of Cl = 20 for O_2.	Amount absorbed (c.c.) by 1 liter of sea-water saturated with air at same temperature.	Absorption coefficient of sea-water of Cl = 20 for N_2 (+argon, etc.).	Amount absorbed by 1 liter of sea-water at same temperature, shaken with air.
0	0.03825	7.97	0.0181	14.31
4	.0346	7.23	.01675	13.24
8	.03167	6.62	.0156	12.34
12	.02923	6.11	.01463	11.57
16	.02722	5.09	.01381	10.92
20	.0254	5.31	.0131	10.36
24	.02368	4.95	.01248	9.87
28	.021	4.62	.01194	9.44
30	.0213	4.46	.01168	9.24

The only time the electric heating failed to be sufficient was on two successive days when we were required to finish the experiments at 30°, although the outdoor temperature was about −30° C. It was then necessary to fill the space between the windows and dark curtains with cotton batting and to turn on the steam heat. The radiator was inclosed in a paper covering and a hole left in this of such size that the temperature did not quite reach 30°, but could be quickly raised to 30° by means of the electric regulation. It was necessary to keep a one-sixth horse-power electric fan and one or two other fans blowing on the apparatus all the time in order to keep all pieces of apparatus at the same temperature. The experiments at 10° were postponed until the outside temperature fell to about −30° C. The experiments at 20° caused little worry in regard to temperature regulation, except on some days when the outside temperature rose to about 10°. Owing to conduction through hollow-tile walls from rooms at about 22°, it was sometimes necessary to leave the window open about an inch. Great care had to be taken then to equalize the temperature in different parts of the room. Several thermometers were compared with a standard and distributed around the room, one or two being placed on every piece of apparatus in order to detect any inequalities in temperature.

Great care was taken to prevent temperature changes in the apparatus. When necessary to handle it, this was done very gingerly, with the tips of the fingers on the extreme ends. Clamps were devised for holding the apparatus in the various necessary positions. During the night the temperature was controlled within 2° of that in the day by means of a thermostat connected with the steam-heat radiator.

HISTORICAL NOTES.

IONIZATION OF SEA-WATER.

Sea-water is a very complex solution, and it is not possible to calculate the concentrations of the different ions in it from the older analyses. Since many of these ions affect the P_H and the CO_2 content, it might be worth while to consider some general facts about the ionization. The simplest method of determining the ionization of a dilute solution of known concentration is by measuring the freezing-point. The accompanying table shows the relation of freezing-point lowering (Δ) to salinity (S) of sea-water from the determinations by H. J. Hansen (Krümmel, I, 241). The ratio of Δ to S is 0.05424 at S = 30 and 0.05492 at S = 40, which might be interpreted to indicate that ionization increases as the concentration increases, but this is only an apparent paradox. In reality, sea-water is a concentrated solution, and it is generally true that the Δ and osmotic pressure of concentrated solutions increase more rapidly than the concentration.

S	Δ	S	Δ
30	1.627	36	1.967
31	1.683	37	2.024
32	1.740	38	2.081
33	1.797	39	2.138
34	1.853	40	2.196
35	1.910		

Electric conductivity experiments show exactly the opposite relation. Figure 23, plotted from the data of Ruppin (Krümmel, I, 291), shows that the conductivity increases less rapidly than the concentration, and that this is almost equally true at various temperatures. Conductivity data must be considered with some caution, however, since viscosity influences conductivity. This is apparent in the change of conductivity with temperature shown in figure 24. It will be noticed that conductivity of sea-water increases very rapidly with temperature, and almost in direct proportion to it. The conductivity is doubled by a rise in temperature of 30°, and it is very improbable that ionization increases so rapidly. Rise in temperature decreases the viscosity of water and in this way increases the conductivity. It is probable that ionization is increased slowly by dilution and also by rise in temperature of sea-water.

THE EXCESS BASE AND THE CO_2 CONTENT OF SEA-WATER.

Tornöe observed that sea-water is alkaline to litmus or rosolic acid. He added a known excess of normal H_2SO_4 to a liter of sea-water, boiled off the CO_2—and caught it in a known solution of $Ba(OH)_2$ for determining the total CO_2—and titrated the sea-water back with CO_2-free alkali, using phenolphthalein as indicator. He found the excess base to be 24 (in our terminology) as a mean value for the parts investigated of the North Atlantic. In titrating with phenolphthalein, the silicates and part of the borates are decomposed (*i. e.*, not re-formed when titrating back), but part of the borates and phosphates act as neutral salts, and we found that the result is about 5 per cent lower than with our method, in which boric and a small amount of phosphoric acid do not affect the result. On the other hand, the results may be too high if too great excess of acid is added or if it is boiled too long, since HCl gas is liable to pass out with the steam. It makes little difference

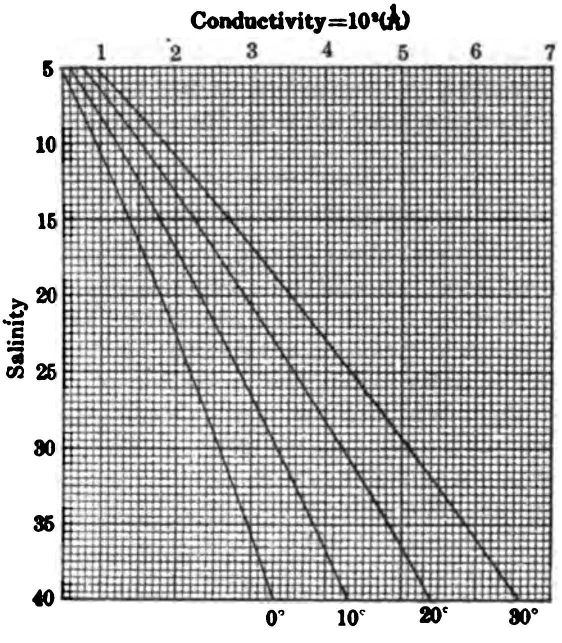


FIG. 23.—Conversion table for finding the electric conductivity of sea-water in hundredths of reciprocal ohms from the salinity and temperature, based on determinations by Ruppin. Each curve is for a different temperature.

whether HCl or H_2SO_4 was added, since HCl is formed in sea-water from H_2SO_4 by dissociation of the large excess of chlorides.

Dittmar found that the average excess base of sea-water was about 25. In a sample of Irish Channel water he found the excess base to be 23 and total CO_2 49.7 c.c. per liter.

Tornöe found that average North Atlantic water contained 49.07 c.c. of CO_2 per liter, and Petterson found that the maximum of all of his samples contained 49.08 c.c. of CO_2 per liter (the water being drawn from a depth of 55 meters). It may be noticed that Tornöe's average is about the maximum, and probably the great majority of samples were from the depths.

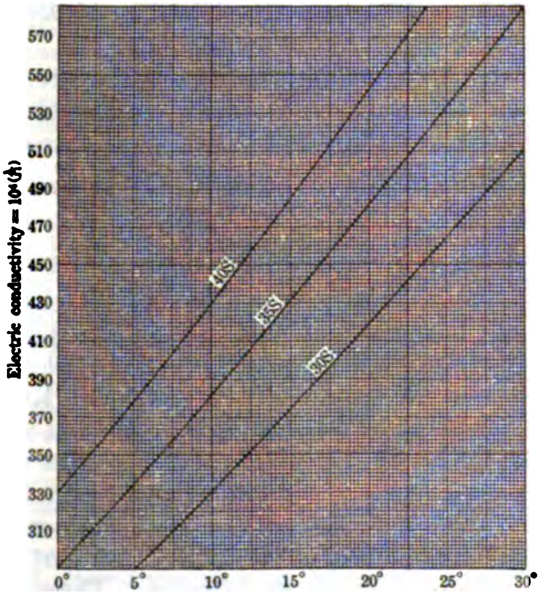


Fig. 24.—Conversion table for finding the electric conductivity in ten-thousandths of a reciprocal ohm from the salinity and temperature. Each curve is for a different salinity.

The samples collected by the *Challenger* during its cruise of three and a half years were boiled by Buchanan and the CO_2 boiled off was estimated; then they were kept several years, during which time they probably absorbed some CO_2 to replace part of that lost; then the total CO_2 they contained was determined by Dittmar and added to Buchanan's figures. Dole found about 45 c.c. of CO_2 per liter in Tortugas sea-water by a titration method.

Dittmar showed that there is an average of 0.44 per cent more lime in the water near the bottom (due to solution of shells) than at the surface of the sea, which accounts for the increase in excess base with depth. The tables of the Danish Ingolf Expedition show the following variations in excess base near the bottom: South of Iceland and east of the barrier (Rykjanaes Ryg) excess base equals 26, west of the barrier it equals 23.7, and in Davis Strait it equals 23.5, the salinity being about 35 and bearing no relation to excess base. The surface-water of Davis Strait averaged 23.6 excess base and 34 salinity. As an exception due to special conditions, Walter and Schirlitz found that the excess base in bottom-water in depressed parts of the Bay of Naples averaged 24, whereas the surface-water averaged nearly 27. Dole found that the excess base in Tortugas water (near surface) varied from 23.7 to 25.7. Ruppin, on the *Poseidon* expedition, found that the excess base averaged 25, with 35 salinity.

Since the excess base or "alkalinity" of sea-water has been expressed in various ways, the following conversion factors are convenient for converting all results to our terminology:

Excess base = 0.8933×c.c. CO_2 in normal carbonate.
= 0.4545×mg. CO_2 in normal carbonate.
= 0.588×mg. OH equivalent to acid used in titration.

The total CO_2 is often given in milligrams, which may be reduced to cubic centimeters by multiplying by 0.509.

CARBON-DIOXIDE TENSION OF SEA-WATER.

We express CO_2 tension in parts per 10,000. If sea-water is bottled it undergoes some fermentation with rise in CO_2 tension, unless an antiseptic is added. Krogh supposed that the addition of 1 gram $HgCl_2$ per liter caused the CO_2 tension to rise 0.35. Krogh found that the CO_2 tension of sea-water was low, except where it was diluted with fresh water from the land. Everywhere it increases with depth. In the Baltic he found tensions as high as 16 at a depth of 95 meters and salinity of 1.3. That the CO_2 tension of the sea-surface is not in equilibrium with the air is indicated by the fact that Krogh found it to vary from 1.5 to 3 in the North Atlantic. These differences were apparently partly due to change in temperature, since, if the water samples were all tested at 12.5°, the tension varied only from 2.05 to 2.95. Perhaps this water came from a uniform source (such as the Gulf Stream) with uniform tension, and the change in temperature on meeting ice and air currents diversified the tension. Since the CO_2 tension of the sea near the Gulf Stream (at Tortugas) varied from 3.3 to 4.7 (calculated from the P_H) and Krogh obtained values as low as 1.5 in the North Atlantic, we may suppose that the water maintains a relatively constant CO_2 content as it flows northward and the lowered temperature decreases the CO_2 tension. This supposition is supported by the fact that Palitzsch found that the P_H is relatively constant, and we have shown that the P_H is dependent on the CO_2 content and not on the temperature. If sea-water at 30° has 4.5 CO_2 tension, simple cooling to 0° will reduce the CO_2 tension to about 1.5.

LITERATURE LIST.

Bang. Allm. Sv. Läkaretidn., 1916, xiii, 549.
Benedict, F. G. Carnegie Inst. Wash. Pub. No. 166, 1912.
Bohr, C. Ann. der Phys. u. Chem., 1899, ccciv (=N. F. lxviii), 500.
Chamberlin, T. C. Jour. Geol., 1899, vii, 682.
Clarke, F. W. U. S. Geol. Survey Bull. 616, 1916 (or 491, 1911).
Clark, W. M., and H. A. Lubs. Jour. Biol. Chem., 1916, xxv, 479.
Dittmar, W. Challenger Reports, Physics and Chemistry, 1884, i (pt. 1), 1.
Dole, R. B. Carnegie Inst. Wash. Pub. No. 182, 1914, 69.
Drew, G. H. Carnegie Inst. Wash. Pub. No. 182, 1914, 7.
Ellis, J. H. Jour. Amer. Chem. Soc., 1916, xxxviii, 737.
Forchhammer, J. G. Phil. Trans., 1865, clv, 203.
Fox, C. J. J. Trans. Faraday Soc., 1909, v, 68.
Haas, A. R. Science, 1916, xliv, 105; Jour. Biol. Chem., xxvi, 515.
Harrison, J. B., and J. Williams. Jour. Amer. Chem. Soc., 1896, xix, 1.
Hasselbalch. Biochem. Zeit., 1913, xlix, 447.
Henderson, L. J. Amer. Jour. Physiol., 1908, xxi, 427.
——— and Cohn. Proc. Nat. Acad. Sci., 1916, ii, 618.
Kendall. Jour. Amer. Chem. Soc., 1916, xxxviii, 1480.
Knudsen. Ingolf Expedition, Hydrography, 153.
Krogh, A. Meddelelser om Grönland, 1904, xxvi, 331, 407.
Krümmel, O. Handb. Oceanographie, Leipsic, 1907, i.
Landolt-Börnstein-Roth. Physikalisch-Chemische Tabellen, Berlin, 1912.
Legendre, R. Compt. rend. Acad., 1906, cxliii, 526.
Lewy, M. Ann. chim. phys., 1852, xxxiv, 5.
Lubs, H. A., and S. F. Acree. Jour. Amer. Chem. Soc., 1916, xxxviii, 2772.
——— and W. M. Clark. Jour. Wash. Acad. Sci., 1915, v, 609; 1916, vi, 481.
Matthews, D. J. Jour. Marine Biol. Assn., 1916, xi, 122.
Mayer, A. G. Am. Jour. Physiol., 1916, xxxix, 375.
McClendon, J. F. Jour. Biol. Chem., 1916, xxviii, 135.
——— and C. A. Magoon. Jour. Biol. Chem., 1916, xxv, 669, (fig. 1).
——— and P. H. Mitchell. Jour. Biol. Chem., 1912, x, 459.
Mines, G. R. Jour. Marine Biol. Assn., 1911, ix, 171.
Murray, J., and J. Hjort. The Depths of the Ocean, London, 1912.
Ostwald, W., and R. Luther. Phys. chem. Messungen, 3d edition, Leipsic, 1910, 438, 465.
Palitzsch, S. Biochem. Z., 1911, xxxvii, 116.
———. Comptes-rendus des travaux du Lab. de Carlsberg, 1916, xi, 199.
Pettersson, O. Scottish Geog. Mag., 1894, 300, 526, 625.
Pouget, I., and D. Chouchak. Bull. Soc. Chim. (4), 1909, v, 104; 1911, ix, 649.
Raben, E. Wissensch. Meeresuntersuchungen Kieler Komm., 1905, viii, 81, 99, 277; 1910, xi, 111, 303, 305.
Regnard. Pettermann's Mitteilungen, 1900, 350.
Ruppin, E. Wissensch. Meeresuntersuchungen Kieler Komm., 1910, xi, 277.
Sörensen, S. P. L. Ergeb. Physiol., 1912, xii, 393.
Tolman, C. F., Jr. Jour. Geol., 1899, vii, 585.
Tornöe, H. Norwegian North Atlantic Expedition, 1876–78; Chemistry, ii.
True, R. H. Science, 1915, xlii, 732.
Veatch, J. A. Proc. Cal. Acad. Sci., 1859 (ser. 1), ii, 7.
Walther and Schirlitz. Z. deutsch. geol. Gesell., 1886, xxxviii, 331.

III.

VARIABILITY OF EGGS AND SPERM OF SEA-URCHINS.

BY A. J. GOLDFARB,
Of the College of the City of New York.

VARIABILITY OF EGGS AND SPERM OF SEA-URCHINS.

By A. J. Goldfarb.

INTRODUCTION.

A clear understanding of the variability in normal fresh eggs and sperm is necessary in order to appreciate and to evaluate the changes that take place in overripe germ-cells. This paper will deal exclusively with the qualitative and quantitative differences of such freshly collected sea-urchin eggs and sperm and with the differences in their early development; it will also furnish a basis for the consideration of the chemical and physical changes in aging or overripe germ-cells in later studies.

To the directors of the laboratories at Woods Hole and Tortugas I wish to extend my thanks for affording me the opportunity to carry on these researches.

TECHNIQUE.

Three different sea-urchins were used, *Toxopneustes* and *Hipponoe* at Dry Tortugas and *Arbacia* at Woods Hole.

The technique is a modification of Lillie's. As soon as possible after the urchins were collected, the eggs were removed, either by inverting each washed and partly dried individual or by removing the ovaries into separate dishes. The eggs were washed two or three times, allowed to settle by gravity in graduates, and finally enough fresh sea-water was added to make a given concentration. The dry sperm was collected from each individual separately. From this sperm any required concentration was made immediately before fertilizing the eggs. The time required to make the different suspensions varied from 30 minutes to 2 hours.

In a typical experiment 5 drops of a standard egg suspension was added to 10 c.c. of filtered sea-water and fertilized with 1 drop (0.05 c.c.) of standard sperm suspension.

The following characters were studied in some detail: (1) Size of eggs. (2) Shape of eggs. (3) Number of eggs with and without jelly layer. (4) Rate of membrane formation. (5) Number and rate of cleaving eggs.

The first three are matters of direct observation; the last two require a word of explanation, for I have found that such matters of detail are significant in these studies. The eggs were shaken and several fields were counted, totaling 100 to 200 or more eggs. At least two counts were made, the first when most of the eggs were in the 2-cell stage—*i. e.*, 1 hour—and the second when most eggs were in the 4- or 8-cell stage—*i. e.*, 2 hours. Preliminary experiments showed that there was little or no increase in total cleavage after the second hour in *Toxopneustes* and *Arbacia*. Therefore the number of eggs that had cleaved in 2 hours was taken as the maximum. In many instances a count was made 40 minutes after fertilization, when the eggs first began to cleave. These three counts afforded the bases for computing the rate of early cleavage and the total cleavage, and also served as a check upon a possible error in the previous count.

Most of the experiments are referred to by date. Thus 7/5 means that the experiment was made on July 5, 1916.

CONCENTRATION OF THE GERM-CELLS.

It was important to determine not only whether differences in concentration of the germ-cells made for differences in membrane formation, in cleavage, etc., but also to determine what concentration gave optimum results for each species of sea-urchin. This was done in the following preliminary experiments which consisted in varying the quantity (1) of eggs of a given female, (2) of sperm, and (3) of sea-water. Tables 1 and 2 give the results of a few such experiments, with so-called "good" and "poor" eggs.

TOXOPNEUSTES.

With the volume of sea-water and the concentration of sperm constant, and with increasing numbers of eggs, a concentration was finally reached in which the rate of membrane formation, rate of cleavage, and total cleavage was considerably reduced.

In experiment 7/5 this injurious concentration was 1,084 eggs in 10 c.c. of sea-water in a Syracuse dish; 8,800 eggs in 7/8A; 9,120 eggs in 7/7A; 21,920 eggs in 7/13A; 38,400 eggs in 7/9A.

In a few experiments the injury was overcome in part by increasing the volume of sea-water 4 to 80 times; in most experiments the injury was not overcome in this manner. (See 7/7B, 7/8B, 7/13C, 7/9B.)

The decreased cleavage was due in part to insufficiency of sperm. The experiments to test this are in entire accord with those of Lillie. An increase of sperm increased cleavage 300 to 800 per cent. (See 7/8C, 7/9C, 7/13 D and E.) A decrease of sperm with low concentration of eggs did not affect the total cleavage. (See 7/13B.)

The lowered cleavage in high concentration of eggs was largely due to asphyxiation; this was evident in the experiments in which the high concentrations of eggs were gently shaken and the cleavage jumped

TABLE 1.—*Toxopneustes.* *Variation of membrane formation and cleavage caused by increasing the concentration of eggs and sperm.*

Experiment No.	Age in hours.		Concentration of eggs, in drops.	Cleavage.		Volume of sea-water.	No. of eggs per drop.	Fert. memb. first appeared in—
	F.	M.		Time.	P. ct.			
				min.		*c.c.*		*min.*
7/5 A	4	½	1	84	54	10	17	1½
			2	84	63	10	17	1½
			4	84	51	10	17	1½
			8	84	56	10	17	1½
			16	84	50	10	17	1½
			32	84	54	10	17	1½
7/5 B	5	1½	1	140	76	10	17	1
			16	140	71	10	17	1
			32	140	75	10	17	2
			64	140	54	10	17	2
			128	140	40	10	17	2
			256	140	28	10	17	5
7/7 A	2½	2½	1	155	90	10	57	....
			20	155	95	10	57	....
			40	155	96	10	57	....
			80	155	90	10	57	....
			160	155	85	10	57	....
			320	155	40	10	57	....
7/7 B	3	2	40	155	90	20	[1]57	....
			80	155	64	40	57	....
			160	155	50	80	57	....
			320	155	12	160	57	....
7/8 A	2	2	1	120	37	10	[2]110	....
			40	120	54	10	[2]110	....
			80	120	33	10	[2]110	....
			160	120	25	10	[2]110	....
			320	120	13	10	[2]110	....
			640	120	7	10	[2]110	....
7/8 B			160	120	25	40	110	....
			320	120	5	80	110	....
			640	120	7	160	110	....
7/8 C			160	120	20	40	[3]110	....
			320	120	44	80	[4]110	....
7/13 A	2	2	1	120	96	10	137	....
			40	120	96	10	137	....
			80	120	90	10	137	....
			160	120	94	10	137	....
			320	120	88	10	137	....
			640	120	58	10	137	....

Experiment No.	Age in hours.		Concentration of eggs, in drops.	Cleavage.		Volume of sea-water.	No. of eggs per drop.
	F.	M.		Time.	P.ct.		
				min.		*c.c.*	
7/13 B	2	2	1	120	92	5	137
			1	120	92	10	137
			1	120	95	20	137
			1	120	96	40	137
			1	120	97	80	137
			1	120	91	160	137
			1	120	97	320	137
7/13 C			1	120	81	5	137
			40	120	87	200	137
			80	120	91	400	137
			160	120	71	950	137
7/13 D			1	120	100	5	[5]137
			320	120	96	5	[3]137
			640	120	94	5	[6]137
7/13 E	3	3	80	120	96	5	[5]137
			160	120	90	5	[3]137
			320	120	85	5	[3]137
			640	120	20	5	[6]137
			1280	120	3	5	[7]137
7/13 F	4	4	25	120	40	5	[5]137
			1600	120	7	5	[5]137
7/14 A	1	1	1	120	100	[8]10	.....
			160	120	95	[8]10	.....
			320	120	95	[8]10	.....
			640	120	90	[8]10	.....
			1280	120	80	[8]10	.....
7/14 B			1	120	100	[9]10	.....
			160	120	100	[9]10	.....
			320	120	95	[9]10	.....
			640	120	95	[9]10	.....
			1280	120	50	[9]10	.....
7/14 C			1	120	100	[10]10	.....
			160	120	95	[10]10	.....
			320	120	95	[10]10	.....
			640	120	95	[10]10	.....
			1280	120	90	[10]10	.....
7/14 D			1	120	100	[11]10	.....
			160	120	95	[11]10	.....
			320	120	95	[11]10	.....
			640	120	95	[11]10	.....
			1280	120	90	[11]10	.....

[1] Area of dishes same as above.

[2] The per cent cleavage at 120 minutes includes only normally cleaving eggs. Hence the lower figures.

[3] Used 2 drops of sperm suspension.

[4] Used 4 drops of sperm suspension.

[5] Used 1 drop of sperm suspension.

[6] Used 3 drops of sperm suspension.

[7] Used 6 drops of sperm suspension.

[8] Shaken gently and frequently.

[9] Five drops of culture removed to 10 c.c. of sea-water, 15 minutes after fertilization.

[10] Same removed 30 minutes after fertilization.

[11] Same removed 45 minutes after fertilization.

from 0 or 1 per cent to 80 per cent. In other experiments when a few drops of highly concentrated suspension were removed to 10 c.c. of sea-water, almost maximal cleavage occurred 15, 30, and 45 minutes after fertilization. (See 7/14, B, C, D.)

In *Toxopneustes*, therefore, optimum results were obtained when the egg concentration was below 8,000 in 10 c.c. of sea-water in a Syracuse dish and when fertilized by 1 drop of standard 0.05 per cent sperm suspension. In actual experimentation about one-tenth of this concentration of eggs was used, and in all experiments the sea-water was doubly filtered and stored in large quantities, to insure greater uniformity and to guard against evaporation.

HIPPONOE.

In *Hipponoe* the results were essentially similar, except that to obtain optimum results much greater concentration of sperm was necessary with a given concentration of eggs. In experiment 6/16 1 drop of 3 times standard sperm suspension fertilized only 3 per

TABLE 2.—*Hipponoe and Arbacia. Variation in cleavage caused by increasing concentration of eggs and sperm.*

Hipponoe.					
Experiment No.	Concentration in drops.		Cleavage.		Volume of sea-water.
	Eggs.	Sperm.	Time.	P. ct.	
			min.		*c.c.*
6/16	1	[1]1	120	3	5
	1	[1]2	120	14	5
	1	[1]4	120	14	5
	1	[1]8	120	29	5
	1	[1]16	120	46	5
6/17	1	[1]1	180	1	5
	1	[1]2	180	14	5
	1	[1]4	180	40	5
	1	[1]8	180	62	5
	1	[1]16	180	75	5
6/18	1	(2)	180	85	5
	1	(3)	180	98	5
	1	(4)	180	78	5
	1	(5)	180	27	5
	1	(6)	180	1	5
	1	(7)	180	1	5

Arbacia.					
Experiment No.	Concentration in drops.		Cleavage.		Volume of sea-water.
	Eggs.	Sperm.	Time.	P. ct.	
			min.		*c.c.*
7/28 A	1	[1]1	120	80	5
	1	[1]$\frac{1}{4}$	120	75	5
	1	[1]$\frac{1}{8}$	120	66	5
	1	[1]$\frac{1}{16}$	120	67	5
	1	[1]$\frac{1}{32}$	120	47	5
	1	[1]$\frac{1}{64}$	120	35	5
	1	[1]$\frac{1}{128}$	120	0	5
7/28 B	1	[1]1	120	83	5
	1	[1]$\frac{1}{16}$	120	46	5
8/9	1	[1]1	120	45	5
	1	[1]$\frac{1}{16}$	120	0	5

[1] Standard sperm suspension.
[2] 1 drop dry sperm in 2 c.c. of sea-water. Used 3 drops of this culture for fertilization.
[3] 1 drop dry sperm in 4 c.c. of sea-water. Used 3 drops of this culture for fertilization.
[4] 1 drop dry sperm in 8 c.c. of sea-water. Used 3 drops of this culture for fertilization.
[5] 1 drop dry sperm in 16 c.c. of sea-water. Used 3 drops of this culture for fertilization.
[6] 1 drop dry sperm in 32 c.c. of sea-water. Used 3 drops of this culture for fertilization.
[7] 1 drop dry sperm in 64 c.c. of sea-water. Used 3 drops of this culture for fertilization.

cent of the eggs, 2 drops only 14 per cent, 8 drops 20 per cent, and 16 drops 46 per cent. The increase was roughly proportional to the increase in sperm. In 6/17B 1 drop of standard sperm suspension gave only 1 per cent cleavage, 16 drops caused 75 per cent to cleave. In 6/17A maximum cleavage occurred with 8 drops of sperm suspension; 16 drops of the same suspension made the solution milky. The matter was tested in other ways. All observations pointed to the conclusion that 15 to 80 times as much sperm was required to produce optimum results in *Hipponoe* as in *Toxopneustes*.

ARBACIA.

F. R. Lillie's careful researches with *Arbacia* have made known the optimum concentrations for this species. My observations corroborate his in every detail. Having ascertained the optimum conditions of concentration for a given surface and volume of sea-water, for each species of egg, I then undertook the study of their normal variability, which will be considered under the following headings: size and shape; jelly layer; membrane formation; cleavage.

VARIATION IN SIZE AND SHAPE OF EGGS.

In a sample lot, eggs were measured consecutively with an eye-piece micrometer (1/3 eye-piece, 1/6 objective); much care was exercised to avoid changes in size from pressure of cover-glass and to avoid errors due to poor focusing. The readings give relative values only. Repeated measurements gave so nearly the same readings that the small number of eggs cited in the table may be taken as a very close approximation to the actual situation.

Table 3 shows the extent of variation in size in the eggs of *Hipponoe*. It shows the considerable variation in the maximum, minimum, and modal size of batches of eggs from different females collected at the same time. The variation, often as great as in females collected at different times, is a result either of a general enlargement or less frequently of a shrinkage from the norm of the species. Such change in size indicates so-called "poor" eggs and is correlated with other evidences of poor physiological condition.

Similar results were obtained with *Arbacia* eggs, though the maximum mode and minimum are different.

SHAPE OF EGGS.

I have no actual counts of the variation in the shape of the eggs. Most fresh eggs are globular, though not infrequently a considerable number of the eggs are distinctly elliptical, in rare instances angular. While I have no records of the numerical relation of these three types of eggs, which intergrade, yet the presence of these three groups and the considerable variation in different females is undoubted.

VARIATIONS IN JELLY LAYER.

With India-ink solutions as used by F. Lillie, the jelly layer surrounding the egg is rendered distinctly visible and the eggs with the thick jelly coating may be readily distinguished from those with either very thin coats of jelly or no jelly. Table 3 gives the percentage of eggs with jelly envelope for each female. Counts of not less than 100 eggs were made. The number of eggs with jelly layers is usually very large: 8 out of 14 females had over 90 per cent with jelly layers; in experiment 6/28, both females had 100 per cent; in experiment 6/27 they varied from 80 to 96 per cent; in experiment 6/25 one female had 100 per cent, the other as low as 63 per cent. It will later be shown that these variations in size and jelly layer in different females also serve as indices of the physiologic condition of the eggs.

TABLE 3.—*Variation in size and jelly of eggs of different females of Hipponoe and Arbacia.*

Hipponoe.													
Date.	No.	Age of eggs.	Diameter of eggs in eye-piece micrometer readings.										Percentage of eggs with jelly.
			21	20.5	20.0	19.5	19.0	18.5	18.0	17.5	17.0	16.5	
		hours.											
6/25	1	2	...	...	2	7	1	...	...	...	...	...	100
	2	2	...	...	...	1	9	1	...	...	...	...	63
	3	5	...	...	...	1	8	1	...	...	...	...	...
	4	0	...	...	...	4	5	...	...	...	...	...	100
6/26	3	0	...	1	3	6	3	...	...	...	...	...	86
	4	0	...	...	8	3	...	...	...	...	...	...	95
	5	0	...	...	5	10	...	...	...	...	...	...	99
	6	0	...	...	...	...	4	8	...	...	...	...	90
6/27	1	3	...	...	1	12	3	...	...	...	...	...	96
	2	3	...	...	...	11	4	...	...	...	...	...	89
	3	3	...	1	10	5	3	...	...	...	...	...	83
	4	3	2	4	3	6	...	...	...	...	...	...	80
6/28	5	½	...	...	...	1	7	4	...	...	...	...	100
	6	½	...	...	2	5	6	2	...	...	...	...	100
Total...........			2	6	34	72	54	16	0	...	...	...	
Arbacia.													
8/17	1	0	...	...	...	...	...	3	8	7	...	...	89
8/10	1	0	...	...	...	...	3	6	5	...	...	...	72
8/11	1	0	...	...	...	...	...	1	3	4	4	1	59
8/28	1	0	...	...	...	...	...	...	...	...	...	...	81

Similar results were obtained for *Arbacia.* It became manifest that one must be cautious in interpreting the results, for with high concentration of sperm the percentage of jelly envelopes was often reduced. For example, in one experiment high concentration of sperm reduced the percentage of jelly by 22 per cent, 13 per cent in another, and 3 per cent in a third.

VARIATION IN MEMBRANE FORMATION.

In order to determine the normal variability in the rate of fertilization-membrane formation, it was important to ascertain the degree of variability due to differences in different samples of sperm and that due to differences in different batches of eggs. A number of the experiments to test this point are given in table 4.

TABLE 4.—*Variation in rate of membrane formation in eggs from different females.*

Date.	No. of F.	No. of M.	Time required for membranes to appear.	Percentage of cleavage in one hour.
			min.	
Toxopneustes:				
7/1	1	1	6	34
	2	1	4	71
	3	1	4	16
	1	2	(1)	24
	2	2	3½	24
	3	2	(1)	9
	1	3	(1)	20
	2	3	3½	95
	3	3	(1)	33
7/9	1	5	3	15
	2	5	4	40
	3	5	3	11
	4	5	(1)	1
7/3	1	1	(1)	80
	1	2	(1)	23
	1	3	(1)	35
	1	4	(1)	68
	1	5	(1)	43
7/2	1	1	3	..
	2	1	4	..
	3	1	3	..
	4	1	(1)	..
7/7	1	1	2	91
	2	1	3	41
	3	1	2½	67
	4	1	3½	86
7/13	1	1	2	99
	2	1	½	83
	3	1	1½	98
	4	1	1½	99

Date.	No. of F.	No. of M.	Time required for membranes to appear.	Percentage of cleavage in one hour.
			min.	
Toxopneustes:				
7/13	1	2	(1)	65
	2	2	2½	48
	3	2	1¼	98
	4	2	1	100
	1	3	..	61
	2	3	3½	48
	3	3	1⅙	85
	4	3	1	81
7/14	1	1	3½	92
	2	1	1½	47
	3	1	1¼	98
7/16	1	1	(1)	50
	2	1	(1)	74
	3	1	2½	81
	4	1	2½	96
	5	1	(1)	18
	6	1	(1)	14
	7	1	2½	40
7/19	1	1	3	82
	2	1	3	96
	3	1	2	83
	4	1	1½	96
	5	1	1½	98
	6	1	1½	94
Arbacia:				
7/28	1	1	(1)	89
	2	1	(1)	89
	3	1	1½	92
	4	1	2	94
	5	1	1½	91
	6	1	1½	98
	7	1	1½	97
	8	1	1½	96

[1] No membranes appeared in first 10 minutes.

When females Nos. 1, 2, and 3 in experiment 7/1 were fertilized by male No. 1, fertilization membranes first appeared in 6, 4, and 4 minutes respectively. The same females tested at the same time by male No. 2 produced membranes in 3½ minutes in one female, and none in the other two females. Male No. 3 gave the same results as No. 2. In experiments 7/13 the membranes first appeared in 30 seconds to 3½ minutes. With other males fertilized at the same time, eggs from the same females formed no membrane at all. Correspondingly wide variations occurred in the other experiments (6/9, 7/3, 7/2, 7/19).

Eggs that form membranes rapidly by one male may develop them slowly by another of the same age and apparently similar condition; but usually the rate of membrane formation is conditioned by the eggs. While the rate may vary with different males, yet each female tends to keep the same relative rate—*i. e.*, there are slow-membrane-forming females and rapid-membrane-forming females.

In every experiment where different females were fertilized by the same male marked differences in the rate of membrane formation occurred. The correlation with cleavage on the one hand and jelly layers and size of the eggs on the other will be given later.

Since the rate of membrane formation varies with different males and with different females under the same experimental conditions, any comparison of results of one group of eggs with another must be made with great caution.

VARIATION IN CLEAVAGE.

Cleavage affords a definite, finely graded, and practical means of measuring variation in the eggs of different females. I have therefore given considerable attention to the variations in rate and in total cleavage in all three sea-urchins.

TOXOPNEUSTES.

The total cleavage is dependent upon the kind of male as well as the kind of female, just as we have observed in the case of the fertilization membrane. For example, 5 samples of the eggs of a given female were fertilized by 5 different males; the percentage that cleaved in 1 hour in these 5 samples was 80, 23, 35, 68, and 43, respectively, a range of 57 per cent. (See table 5, experiment 7/3.)

When different samples of eggs of a single female were fertilized by the same male the maximum difference was within 5 per cent—*i. e.*, the experimental error was below 5 per cent.

When different females were fertilized by the same male, all suspensions being standardized, the variation in cleavage was surprisingly large. It varied from 1 per cent in experiment 7/2B to 98 per cent in many experiments, 7/21, 7/19, 7/14, 7/12, etc. The range of variability as expressed by the difference between maximum and minimum cleavage for any given experiment in which a given male fertilized different females, was 11, 15, 16, 39, 48, 51, 52, 53, 54, 55, 57, 72, 74, 75, and as large as 87 per cent.

In a second series of experiments different males were used either with samples of a given female or with different females. (See experiments 7/1, 7/13.)

In 7/13, males Nos. 1, 2, 3, and 4 fertilized female No. 1 and gave 99, 83, 98, and 99 per cent cleavage respectively. With female 3, the corresponding males gave 57, 30, 50, and 46 per cent. Female No. 1 gave consistently high cleavage by all the males, and female No. 3 gave consistently low cleavage. As in rate of membrane formation, one may speak of high-cleavage eggs and low-cleavage eggs. Apparent exceptions arise when low-cleaving eggs were fertilized by high-cleavage sperm.

TABLE 5.—*Variation in percentage of cleavage, of eggs, and of sperm from different individuals of Toxopneustes.*

Date.	No. of female.	No. of male.							Age of germ-cells.	Maximum difference in cleavage percentage.
		1	2	3	4	5	6	7		
									hours.	
7/3	1	80	..	..	..	..	..	..	½	....
	2	23	..	..	..	..	..	..	½	....
	3	35	..	..	..	..	..	..	½	....
	4	68	..	..	..	..	..	..	½	....
	5	43	..	..	..	..	..	..	½	....
		[1]57	..	..	..	..	..	..	..	....
7/2 to 7/21	1	15	50	98	92	81	82	51	¼	....
	2	40	43	98	47	96	96	98	¼	....
	3	11	95	87	98	18	83	11	¼	....
	4	1	80	..	..	14	96	..	1	....
	5	..	..	..	..	40	98	..	2	....
	6	..	..	..	..	..	94	..	1	....
		[1]39	52	11	51	72	16	87	..	....
7/1	1	34	24	20	..	..	..	..	1	14
	2	71	24	95	..	..	..	..	1	71
	3	16	9	33	..	..	..	..	1	24
		[1]55	15	75	..	..	..	..	..	..
7/13	1	99	83	98	99	..	..	..	½	16
	2	25	64	93	100	..	..	..	½	75
	3	57	30	50	46	..	..	..	½	27
		[1]74	53	48	54	..	..	..	..	..

[1] Maximum difference.

Since the variability in the reactions between these fresh eggs and sperm is so amazingly large, one should be cautious about comparisons of fertilized eggs from different individual sea-urchins, even when of one and the same species; yet this error is commonly made.

TABLE 6.—*Variation in cleavage of germ-cells of different individuals—Hipponoe.*

Date.	No. of female.	No. of male.	Age of germ-cells. Female.	Age of germ-cells. Male.	Per cent cleavage.	Maximum difference in cleavage percentage.	Time since fertilization.
			hours.	*hours.*			*hours.*
6/21	1	1	0	0	45	....	3
	1	2			76	31	
6/23	1	1	0	0	16	....	2
	2	1			6	10	
6/27	1	1	0	0	91	....	1⅓
	2	1			12	....	
	3	1			85	....	
	4	1			8	83	
6/26	1	1	0	0	83	....	2
	2	1			66	17	
7/1	1	1	0	0	90	....	1⅓
	2	1			54	....	
	3	1			59	....	
	4	1			47	....	
	5	1			16	94	
7/2	1	1	0	0	56	....	1⅓
	2	1			51	....	
	3	1			74	....	
	4	1			57	....	
	5	1			70	....	
	6	1			27	47	
7/6	1	1	0	0	10	....	1⅓
	2	1			37	....	
	3	1			19	....	
	4	1			64	54	
7/16	1	1	0	0	0	....	2
	1	2			0	....	
	1	3			0	....	
	3	4			44	....	
	3	5			0	....	
	3	6			8	44	
6/29	1	1	0	0	19	....	2⅓
	2	1			94	....	
	3	1			73	75	
6/29	4	5	1⅔	1⅔	92	....	1⅓
	5	5			97	....	
	6	5			83	14	
7/8	1	1	0	0	52	....	1⅔
	2	1			65	....	
	3	1			58	....	
	4	1			41	24	
7/8	5	1	2¼	2¼	92	....	1⅓
	6	1			22	....	
	7	1			38	....	
	8	1			77	70	
7/3	1	1	0	3	62	....	2
	2	1			94	....	
	3	1			13	81	
6/28	1	1	0	7	24	....	1⅓
	1	2			57	33	
7/30	1	1	⅓	⅓	71	....	1⅓
	2	1			76	5	

HIPPONOE.

The variation in cleavage of *Hipponoe* eggs was essentially the same as in *Toxopneustes*. The data are brought together in table 6.

Samples of eggs of a given female fertilized by different males gave 45 and 76 per cent cleavage, respectively, a range of 31 per cent. (Experiment 6/21.)

Eggs of different females fertilized by the same male varied from 5 to 81 per cent in the 13 experiments cited in table 6. The maximal difference in each experiment was 5, 9, 10, 17, 24, 47, 54 per cent and as large as 70, 74, 75, and even 81 and 83 per cent.

In these experiments so called "good," "poor," and "bad" eggs were used. These terms will later be defined in definite chemico-physical entities.

TABLE 7.—*Variation in cleavage of eggs and sperm of different Arbacia individuals.*

Experiment No.	Age of germ-cells.		No. of male.	Egg No. 1.	Egg No. 2.	Egg No. 3.	Egg No. 4.	Egg No. 5.	Egg No. 6.	Egg No. 7.	Egg No. 8.	Maximum difference (per cent).
	Female.	Male.										
8/12..........	4½	4½	1	81	58	1	77	91	..	..	..	90
	4⅓	4⅓	2	72	69	4	62	53	..	..	..	68
8/13..........	23	23	1	75	70	3	0	74	..	..	..	75
8/14..........	1⅓	1⅓	1	80	74	99	96	100	..	..	..	26
	5	5	5	93	90	100	85	95	..	..	..	15
8/16..........	1⅓	1⅓	1	100	98	100	88	98	92	100	..	12
7/28 (1 hour)...	0	0	1	83	89	92	94	91	98	97	96	15
7/28 (2 hours)..	0	0	1	92	98	97	97	98	97	99	99	7
8/2...........	0	0	1	100	100	..	..	..	..	..	..	0

ARBACIA.

A few observations were made upon *Arbacia*, which live in much deeper and colder waters. The results are shown in table 7.

In experiment 8/2, table 7, eggs of different females fertilized by the one male gave 100 per cent cleavage for each. In experiment 7/28 all 8 females gave a high cleavage; the maximum difference was 15 per cent at end of 1 hour and only 7 per cent at end of 2 hours. In the other experiments the range of variability was much greater—12, 15, 26, 68, 75, and 90 per cent in different experiments. There were low-cleaving females (see experiments 8/12 and 8/13); others were sterile or practically so (see 8/12 and 8/13); others high-cleaving females. The eggs appeared alike, yet upon actual test they showed a very marked range of variability. On some days all the females seemed to be high-cleaving females, as in 7/28; on other days there was marked variation, as in 8/12, 8/13, etc.

CORRELATION OF THE DIFFERENT VARIATIONS.

These observations and experiments furnished unmistakable evidence of a high correlation of these variants in all three sea-urchins. From table 3 the size of eggs and the percentage of eggs with jelly envelopes may be compared; also in table 4 the rate of membrane formation and total cleavage. From the different experiments it was observed that the following characters and characteristics were closely correlated, namely:

No. 1. Globular shape of all or nearly all of the eggs.
No. 2. Large percentage of the eggs with the jelly envelope.
No. 3. Size of the eggs close to mode, with very little variation.
No. 4. (*a*) Fertilization membrane formed in about 2 minutes.
  (*b*) Large percentage of the eggs form a membrane.
No. 5. (*a*) A rapid rate of first cleavage.
  (*b*) A high percentage of cleavage.

Vice versa, the following were closely correlated:

No. 1. A *large* percentage of *elliptical* eggs.
No. 2. A *low* percentage of eggs with their *jelly* envelopes.
No. 3. Eggs usually larger than mode, and much variation.
No. 4. (*a*) Fertilization membranes slow to appear.
  (*b*) Small percentage of eggs form membranes.
No. 5. (*a*) Slow rate of first cleavage.
  (*b*) Low percentage of cleaving eggs.

DISCUSSION.

An adequate understanding of these variations can not be had without a knowledge of the results of experiments with aging germ-cells. It is expected that such results will appear in a later publication, but at present I wish merely to point out the nature of some of the variants and to call attention to the marked range of differences in each of the variants in *fresh germ-cells*. If these facts are thoroughly appreciated, not only will many conflicting results on the physiology of eggs and sperm disappear, but a clearer understanding of the situation may result.

A number of investigators have remarked on the fact that under apparently identical conditions varying experimental results were obtained, and have suggested that the differences were due to some physiologic difference in the eggs.

Stockard (7) stated "the fact that a number of eggs when subjected to the same solutions do not all respond in a like manner" was due to "*differences in individual resistance and vigor*."[1]

F. R. Lillie (2) noted that "the failure to obtain exactly the same curve * * * was due in part to the *natural variability of different lots* of eggs and sperm." Elsewhere he asserts that the percentage of cleavage was more *dependent on the conditions of the sperm* than upon its concentration.

---

[1] All italics are mine.

J. Loeb and H. Wasteneys (5) believed that the different results on the effects of weak and strong bases on the oxidation of eggs of the sea-urchin may be due to *differences in the eggs.*

Wasteneys (8) later, in working upon the oxidation of sea-urchin eggs, concluded that the variations in his results were due to *differences in sensitiveness of eggs of different females* and perhaps to differences of temperature.

R. S. Lillie (3), from a study of the rate of swelling of eggs in diluted sea-water, also concluded that the variability in rate was due to the *condition of the eggs.*

In studying the fertilizin phenomenon, F. R. Lillie (1) arrived at a more adequate appreciation of the nature of this variability when he held that the *condition of the eggs*, whether ripe or immature, fresh or stale, with or without jelly, is more important than concentration of the eggs. Elsewhere (p. 568) he remarked that "*the condition of the gonads* is the most variable thing in summer sea-urchins."

R. S. Lillie (3) made a notable contribution when he concluded that resistance to osmotic disruption in dilute sea-water was "a convenient index of the physiologic condition of the plasma membrane" and that "an intimate connection exists between the general physiologic condition of the egg and the physiologic state of the plasma membrane."

We are not justified in assuming that all ripe eggs freshly removed from the bodies of the sea-urchins are in a uniform or nearly uniform physiologic condition or that at a subsequent interval in sea-water the eggs continue to remain in a fairly uniform physiologic condition.

This study and later ones have forced upon me the conclusion that these assumptions are without basis. Eggs freshly removed from the body are *not in the same physiologic condition*, as measured by any of the tests I have proposed. Given an originally limited variability of the eggs—for the chemico-physical explanation of which see Loeb and Chamberlain (4)—which variability I shall term the primary variability, further variations and types of variants are superimposed by reason of the fact that the eggs do not all ripen[1] at the same time within the body. Further variability is due to the fact that the eggs are subjected to the injurious influences of the body fluid for intervals varying with the time since maturation.

When such eggs are placed in sea-water the physiologically different groups of eggs are again affected very unequally.

The totality of these varying influences produces marked variations of a number of characters among the eggs of a given female, as well as the eggs of different females.

We have no direct or simple measure of the time since maturation of the egg, nor of the physiological condition of the eggs. But one can obtain a very definite idea of the physiological condition of any sample of eggs by ascertaining the extent of certain changes in size, shape, dissolution of jelly layer, retardation of rate of membrane formation, and rate of cleavage and total cleavage, all of which, and possibly others, serve as different and corroborative indices of their physiological condition. The nature of the chemico-physical factors involved in these variations was made known from a study of aging eggs.

---

[1] Eggs are ripe in this sense at the moment when germinal vesicle breaks down.

From this it follows that for a study of the physiology of the germ-cells, or for any experimental work that involves a comparison of germ-cells from different individuals, it is not sufficient to use eggs of the same chronologic age, but by actual tests, as shown above, to choose eggs of the same physiologic age—*i. e.*, eggs most nearly in the same physiologic condition. If this be done we should expect less conflicting results than have obtained heretofore.

CONCLUSIONS.

1. The optimum conditions of temperature, volume of sea-water, surface exposure, and concentration of eggs and of sperm were ascertained.

2. The eggs and sperm used in these experiments *were immediately removed from freshly collected sea-urchins.*

3. Three species of sea-urchins were used, *Toxopneustes* and *Hipponoe* of shallow tropical waters and *Arbacia* of deeper, colder waters. The fundamental results for all these were in entire accord.

4. The *Arbacia* germ-cells are less variable than either of the tropical forms.

5. Variability, with respect to cleavage, differs with different individuals. The eggs of one female when fertilized by a given male may give markedly different percentages of cleaving eggs when fertilized by a second male. The difference is usually much greater, all other conditions remaining constant, when eggs of different females are fertilized by the same male. The relative difference is usually constant. Some females have a high percentage of cleaving eggs; other females a low percentage. Cleavage is a function of the kind of eggs used.

6. The variations studied were: size and shape of eggs; presence of jelly envelope; membrane formation; cleavage.

7. There is a surprising range of variability for each of these characters and characteristics, less for size or shape, greater for jelly and membrane formation, and greatest for cleavage.

8. The eggs of some females showed little variation in size from the norm, were usually globular, and a large proportion had the characteristic jelly envelope. These eggs were in good physiologic condition.

9. The eggs of other females varied considerably in size from the norm, had a high percentage of elliptical eggs and a low percentage of intact jelly envelopes. These eggs were in poor physiologic condition.

10. In some females the eggs formed fertilization membranes in about 2 minutes, others required 3 to 6 minutes, or formed no membranes at all. Rapid membrane formation was associated with small variability of size, globular shape, and high jelly count; slow formation with enlargement or considerable variation in size, high percentage of elliptical eggs and low jelly count. The former were in good, the latter in poor physiologic condition.

11. The rate of early cleavage and the total cleavage varied most widely. The total cleavage varied from complete sterility to 100 per cent cleavage. High cleavage is correlated with normal size, globular shape, large jelly count, and rapid membrane formation; and *vice versa*, low cleavage with the opposite conditions.

12. This amazingly large variation is due to:

a. A primary, small variability in ripe eggs.
b. The effect of chemico-physical agencies acting on the ripe eggs within the body of the female.
c. A variation in time or ripening of different groups of eggs within a given female.
d. The time between maturation and removal of the eggs to sea-water, which time may be different for different females.
e. The differential effect of sea-water upon physiologically different eggs.

Eggs which have been removed from different individuals at the same time and are of the same chronologic age are not necessarily in the same physiologic condition. The extent of the change may be determined by the study of their variations.

13. For certain experimental work the eggs should be grouped, not according to age—*i. e.*, the time since removal from the body—but according to their physiologic state, which may be accurately determined by the various tests suggested above.

BIBLIOGRAPHY.

1. Lillie, F. R. 1914. Studies of fertilisation. VI. The mechanism of fertilisation in *Arbacia*. Jour. Exper. Zool., vol. 16, 523.
2. ———. 1915. Studies of fertilisation. VII. Analysis of variations in the fertilising power of sperm suspensions of *Arbacia*. Biol. Bull. 28, 228.
3. Lillie, R. S. 1916. Physiology of cell division. VI. Rhythmical changes in resistance of dividing sea-urchin eggs to hypotonic sea-water. Jour. Exper. Zool., vol. 21, 369.
4. Loeb, J., and M. M. Chamberlain. 1915. An attempt at a physico-chemical explanation of certain groups of fluctuating variations. Jour. Exper. Zool., vol. 19, 559.
5. ———, and H. Wasteneys. 1915. Further experiments on the relative effect of weak and strong bases on the rate of oxidation in the egg of the sea-urchin. Jour. Biol. Chem., vol. 21, 153.
6. ———. 1913. Artificial parthenogenesis.
7. Stockard, C. R. 1913. Experimental study of the position of the optic anlage in *Amblystoma punctatum*, with a discussion of certain eye defects. Amer. Jour. Anat., 15.
8. Wasteneys, H. 1916. The rate of oxidations in reversed artificial parthenogenesis. Jour. Biol. Chem., vol. 24, 281.

IV.

ANALYTICAL SEARCH FOR METALS IN TORTUGAS MARINE ORGANISMS.

BY ALEXANDER H. PHILLIPS,
Of Princeton University.

IV.

ANALYTICAL SEARCH FOR METALS IN TORTUGAS MARINE ORGANISMS.

By ALEXANDER H. PHILLIPS,
Of Princeton University.

ANALYTICAL SEARCH FOR METALS IN TORTUGAS MARINE ORGANISMS.

BY ALEXANDER H. PHILLIPS.

INTRODUCTION.

The problem upon which it is hoped that these analyses will cast some light was stated in Year Book No. 14, page 193, of the Carnegie Institution of Washington. A large number of specimens were collected and, while only a few of them have been analyzed for metals, the results justify the present publication, leaving the discussion to some future time when the analyses of all the samples or specimens collected, as well as others from various localities, might be included.

The specimens were dried in the air-bath at 110° C., were ground, and bottled in bottles with tight-fitting stoppers. Some specimens slowly decomposed at 110° C. and constantly lost weight; in such cases the specimens were heated in the bath for 8 hours and then bottled as if dry. The metals determined were iron, manganese, zinc, copper, and lead. For the determination of zinc, copper, and lead, when the dried material was sufficient, 20 grams were used as a sample; when it was not possible, through lack of material, to use 20 grams, the results, as tabulated for convenience of comparison, are all calculated to 20 grams.

METHOD.

The method of analysis followed is that advised by the U. S. Department of Agriculture, Bureau of Chemistry, Bulletin No. 107 (revised), with slight modifications, as tin was not sought. The sample was incinerated in a muffle at a low red heat, not high enough to fuse the chlorides. After solution of the ash in HCl and evaporation to dryness, sulphides were precipitated in a hydrochloric-acid solution. They were then dissolved in HNO_3, when copper and lead, if any, were determined electrolytically in the nitric-acid solution. Iron and phosphoric acid were separated from zinc in the filtrate from the sulphides by the basic-acetate method, when the zinc was precipitated as a sulphide in an acetic-acid solution and weighed as ZnO. If this zinc oxide was found to be impure it was dissolved in hydrochloric acid, filtered, and the filtrate was boiled with an excess of NaOH in platinum, any precipitate filtered off, and the zinc reprecipitated with Na_2CO_3 and again weighed as ZnO.

Iron was estimated in a separate sample, usually 2 grams, by titration with permanganate. Manganese was also estimated in a separate sample, by the colorimetric method, and both, as tabulated below, are calculated to amounts contained in 20 grams of the dry sample. The weights, etc., are given in grams.

Specimen No.	Sample taken for analysis	Weight complete.	Weight of shell.	Weight of tissues.	Weight of liver.
614 B C 1	20 grams of liver	4,365	2,990	1,375	157
614 B C 2	20 grams of liver	3,705	2,595	1,110	105
614 K 1	20 grams of liver	4,265	2,875	1,390	405
614 K 2	20 grams of liver	3,730	2,485	1,245	315
614 R	20 grams of tissues	770	610	160	...
614 A T	20 grams of liver	21,015	17,835	3,180	585
614 A U	5 grams of liver	1,765	1,527	238	57
614 D 2	20 grams of tissues	2,925	2,555	370	...
614 E F G	20 grams of tissues	8,368	7,046	1,322	...
614 L	20 grams of tissues	1,225	787	438	...
614 A S	10 grams	1,515	.....	.....	[1]201
614 A S 1	2 grams of liver	1,515	.....	.....	22
614 A S 2	20 grams of liver	.....	.....	.....	67
615 E	20 grams	.....	.....	.....	1,035
615 E 2a	10 grams[1]	.....	.....	[1]190	.....
614 C B 1	15 grams	450	.....	.....	.....
614 C B 2	20 grams	.....	.....	.....	.....
614 C B 3	3 grams	.....	.....	.....	18.5
614 A 1	20 grams	2,670	.....	1,945	.....
614 A 2	20 grams	2,670	.....	725	.....
614 N	20 grams	2,215	.....	.....	.....
614 B Q	20 grams	395	.....	.....	.....

Specimen No.	Weight dried at 110° C.	Copper.	ZnO	Fe	MnO	PbO_2
614 B C 1	[2]69.2	0.0434	0.0036	0.0121	0.00022	0.00005
614 B C 2	[2]45.1	.0745	.0123	.0208	.00020	.00005
614 K 1	[2]180.8	.0007	.0019	.0108	.00016	....
614 K 2	[2]136	.0019	.0014	.0081	.00008	....
614 R	41.5	.0006	.0014	.0112	.0011	....
614 A T	[2]175.9	.0006	.0019	.0176	.00355	....
614 A U	[2]8.1	.0008	.0076	.0397	.0043	....
614 D 2	81.5	.0025	.0051	.0193	.0021	....
614 E F G	322	.0002	.0038	.0073	.0022	....
614 L	101	.0007	.0077	.0114	.00016	....
614 A S	[1]17.6	.0140	None.	.0175	.0014	....
614 A S 1	4	.0180	.0040	.0057	.000016	....
614 A S 2	31.7	.0222	.0038	.0056	Trace	....
615 E	219	.0034	.0133	.0054	.0001	....
615 E 2a	17.24	.0170	.0014	.0057	.00084	....
614 C B 1	22.4	.00027	.0011	.0079	.0002	....
614 C B 2	92	.0003	.0016	.0087	.0002	....
614 C B 3	5.4	.0022	.0020	.0124	Trace	....
614 A 1	404	Trace	.0043	.0057	Trace	....
614 A 2	74	.0004	.0013	.0002	Trace	....
614 N	215	.0003	.0006	.0235	.0002	....
614 B Q	44	.0004	.0032	.0229	.0002	....

[1] Blood. [2] Liver.

Descriptions of Specimens Analyzed.

614 B C 1. *Fasciolaria gigantea;* horse conch; liver of an immature male.
614 B C 2. *Fasciolaria gigantea;* liver of a much older individual, sex not noted.
614 K 1. *Cassis* (?); queen conch; liver only; sex not noted.
614 K 2. *Cassis* (?); queen conch; liver only; sex not noted.
614 R. *Strombus bituberculatus;* all soft parts of 6 females are represented in the samples analyzed.
614 A T. *Strombus gigas;* represents the livers of 8 individuals of both sexes.
614 A U. *Strombus gigas;* the liver of a much younger individual than any included in 614 A T.
614 D 2. *Strombus gigas;* represents all the soft parts of a single individual with a very old, battered, and thick-lipped shell.
614 E F G. *Strombus gigas;* represents all the soft parts of 3 individuals, all in the first year of maturity.
614 L. *Fulgur perversus;* represents all the soft parts of 4 adult individuals.
614 A S. *Palinurus;* crayfish; represents the blood only.
614 A S 1. *Palinurus;* crayfish; liver of a single individual.
614 A S 2. *Palinurus;* crayfish; liver of a much larger individual.
615 E 1. *Limulus polyphemus;* livers, ovaries, and testes of four individuals, two males and two females, collected at the Marquesas.
615 E 2a. *Limulus polyphemus;* 190 c.c. of blood.
614 C B 1. *Aplysia;* sea cat; one complete individual.
614 C B 2. *Aplysia;* sea cat; five complete individuals.
614 C B 3. *Aplysia;* sea cat; the livers of two individuals.
614 A 1. *Holothuria bermudiana;* muscular tissue, etc., of several large individuals, less the intestines.
614 A 2. *Holothuria bermudiana;* intestines of the individuals in 614 A 1.
614 N. *Ciona atra;* black tunicate, living on the walls of the moat of Fort Jefferson.
614 B Q. A gray tunicate also from the moat of Fort Jefferson.

The constant occurrence of both copper and iron in the analyses is to be expected, but the large amounts of copper in some and its variability, even in individuals of the same species, is to be noted. Zinc is present in all the specimens analyzed except the crayfish blood, and as the sample was exhausted in the first analysis this could not be tested further. Since the Tortugas Islands are far removed from any possible contamination of the sea-water, zinc must be considered as a normal constituent of these forms. Manganese is present in all the specimens analyzed, but varies considerably. The most remarkable occurrence, however, is that of lead in the liver of *Fasciolaria gigantea* in quantity just sufficient to weigh, in a 20-gram sample of dried liver, but quite enough to yield good qualitative tests.

V.

ON THE COMPOSITION OF CASSIOPEA XAMACHANA AND THE CHANGES IN IT AFTER STARVATION.

BY S. HATAI,

Of the Wistar Institute of Anatomy and Biology.

One figure.

ON THE COMPOSITION OF CASSIOPEA XAMACHANA AND THE CHANGES IN IT AFTER STARVATION.

By S. Hatai.

INTRODUCTION.

Mayer (1914) reported that in *Cassiopea* the percentage of nitrogen to the total solids remains constant during the entire period of starvation; he infers that "no appreciable chemical change occurs in the composition of its body, and that there is no appreciable selective use of different substances at different times during the progress of starvation." This is remarkable, since the starving mammalian body reveals a totally different relation, owing to the rapid disappearance of reserve substances, such as carbohydrates and fats, during the earlier period of starvation, followed by a slow consumption of protein substances later. Thus the starving mammalian body gives different percentage values for the nitrogen at different periods of starvation, especially in the earlier stages.

The general anatomy of *Cassiopea* suggests that since by far the greater fraction of the body is represented by the reserve jelly-like substance, while the amount of living cellular elements is small, constancy in the percentage of nitrogen means that practically all the nitrogen is represented by that of the jelly-like substance, which by its relative abundance masks those chemical changes that may occur within the epithelial elements. To test this point the experiments to be described were undertaken.

NORMAL GROWTH OF DIFFERENT PARTS OF THE BODY.

Cassiopea may be divided into three distinct parts: (1) Mouth-organs, (2) umbrella, and (3) velar lobes, which differ not only morphologically, but also in their absolute weights and in the relative amount of cellular and non-cellular constituents. It was, therefore, thought desirable to study the normal growth of these parts in order to determine whether the starving cassiopea loses weight uniformly or whether the loss is dissimilar in the three parts concerned. Altogether 37 normal examples of *Cassiopea* of various body-weights were examined, and the results obtained are given in table 1.

The individual records were plotted, together with the average values, with a view to showing at a glance the extent of variation, and are given in figure 1.

Table 1 shows that the three parts increase in weight with the increase of the entire body-weight, but the relative weights of these parts change as the body becomes heavier. The greatest increase in relative weight is made by the velar lobes, while those made by the mouth-organs and the umbrella follow in the order named. It should be noted that, although the mouth-organs show the greatest weight during most of the animal's life, yet in the earlier period, where the entire body weighs less than 0.5 gram, the umbrella has a greater weight than the mouth-organs.

TABLE 1.—*Different body-weights of the normal Cassiopea, the weights of mouth-organs, umbrella, and velar lobes.*

Entire body.	Mouth-organs.	Umbrella.	Velar lobes.	No. of medusæ.
grams.	*grams.*	*grams.*	*grams.*	
0.098	0.040	0.056	0.002	1
.316	.141	.154	.022	5
.806	.384	.359	.063	4
8.990	5.918	2.550	.521	5
54.219	32.182	18.103	3.933	7
118.313	66.800	42.092	9.421	6
163.993	92.694	59.080	12.219	5
234.284	140.497	78.255	15.531	4

The graphs in figure 1 show that the growth in weight of these three parts on body-weight is almost linear. It would be interesting to determine how the growth curve of *Cassiopea* with respect to age appears, but for the necessary data we must await future investigations.

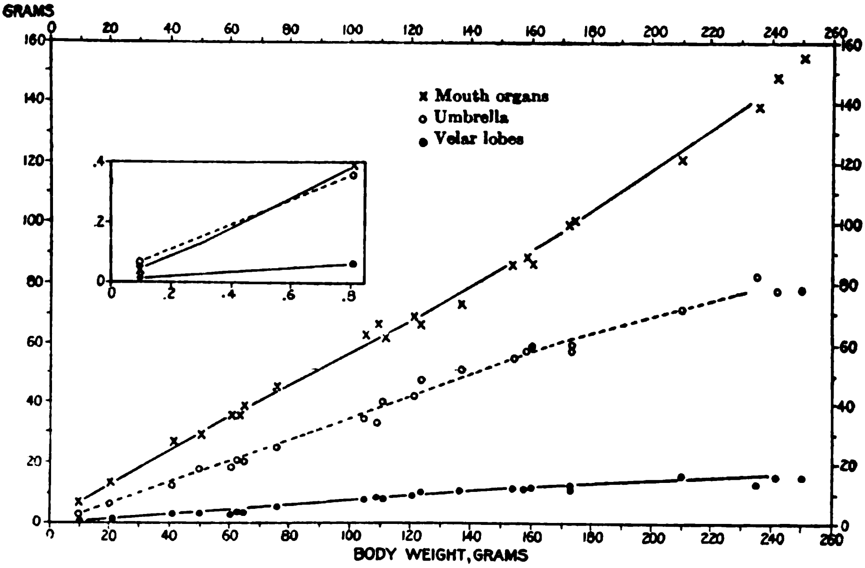


FIG. 1.—Showing the growth of three parts of the body according to the increasing weight of *Cassiopea xamachana*.

ANALYSIS OF ENTIRE BODY.

Several freshly caught cassiopeas were weighed and then placed in the oven for one week at a temperature of 90° to 95° C. The percentages of water and of solids were thus determined and the solids again were analyzed for nitrogen and ash. The results of these determinations are given in table 2.

TABLE 2.—*Fresh weight, dry weight, water, nitrogen, and ash in a series of Cassiopeas of increasing body-weights.*

Body-weight, fresh.	Body-weight, dry.	Water.	Solids.	Total nitrogen.	Nitrogen in solids.	Total ash.	Ash in solids.	Remarks.
		p. ct.	*p. ct.*	*mgm.*	*p. ct.*	*mgm*	*p. ct.*	
0.402	0.023	94.28	5.72	......	.....	...	.....	
1.000	.055	94.50	5.50	......	.....	...	.....	
1.383	.085	93.86	6.14	2.77	3.226	...	.....	Average of 3.
1.540	.085	94.48	5.52	......	.....	...	.....	
1.786	.113	93.68	6.32	3.83	3.402	...	.....	Average of 3.
5.559	.329	94.08	5.92	9.80	2.983	...	.....	Average of 2.
5.876	.333	94.33	5.67	......	.....	174	52.16	
7.496	.454	93.95	6.05	14.56	3.207	...	.....	
8.257	.483	94.15	5.85	13.86	2.870	...	.....	
9.128	.540	94.08	5.92	......	.....	285	52.78	
10.060	.592	94.12	5.88	17.78	3.003	...	.....	
10.931	.625	94.28	5.72	17.40	2.784	...	.....	
10.958	.650	94.07	5.93	......	.....	329	50.62	
20.530	1.194	94.19	5.81	27.16	2.275	...	.....	
		[1]94.14						
100.055	5.401	95.10	4.9	138.59	2.566	...	.....	Mayer, 1914.
109.641	5.43	95.10	4.9	116.58	2.147	...	.....	Mayer, 1914.

[1] Average.

PERCENTAGE OF WATER.

It is interesting to note that the percentage of water is practically identical in all sizes of *Cassiopea* here examined, thus indicating that the jelly-like substance of which the body of *Cassiopea* is largely composed remains nearly unmodified throughout the life cycle. This is in remarkable contrast to the water-content of mammalian bodies at various ages in which the younger individuals give the higher percentage of water and *vice versa* (Hatai, 1916).

PERCENTAGE OF NITROGEN IN TOTAL SOLIDS.

The nitrogen in the total solids was determined by the usual Kjeldahl method and the results are given in table 2. We notice that despite the similarity of the water-content, the percentage of nitrogen in the solids shows a progressive decrease with increasing body-weight. Although this difference in the relative nitrogen-content between the smaller and larger animals is not very large, nevertheless the existence of such a difference can not be doubted. We assume that this difference in the nitrogen-content is due to the relative abundance of cellular elements in the smaller individuals, contrasted with an excess of the reserve materials composed of organic substances poorer in nitrogen in the larger individuals. We thus find that, within the limits taken, the reserve jelly-like substance and cellular elements probably both have the same water-content, since otherwise the same percentage of water throughout the series could not be expected.

PERCENTAGE OF ASH IN SOLIDS.

Since the percentage of water is similar in all the sizes of *Cassiopea* examined, and since both cellular elements and reserve materials have a like water-content, we should anticipate that the ash-content would be also similar in the several stages, and the determination of the ash-content on three individuals supports this conclusion. The proportion of ash is as high as 52 per cent, thus making the organic substances less than half of the entire solids.

ANALYSIS OF DIFFERENT DIVISIONS OF THE BODY.

Using four cassiopeas, which also appear in table 2, the three different parts of the body were separately analyzed. The results are shown in table 3.

Table 3.—*Water-content, solids, and nitrogen in three different parts of the normal Cassiopea.*

Parts.	Weight, fresh.	Weight, dry.	Water.	Solids.	Total nitrogen.	Nitrogen in solids.
	grams.	*grams.*	*p. ct.*	*p. ct.*	*mgm.*	*p. ct.*
Mouth-organs....	4.925	0.311	93.69	6.31	10.50	3.376
Umbrella........	2.133	.118	94.47	5.53	2.66	2.254
Velar lobes......	.438	.025	94.29	5.71	1.40	5.600
	7.496	.454	93.94	6.06	14.56	3.207
Mouth-organs....	5.553	.334	93.99	6.01	9.94	2.976
Umbrella........	2.282	.123	94.61	5.39	2.52	2.048
Velar lobes......	.422	.026	93.84	6.16	1.40	5.384
	8.257	.483	94.15	5.85	13.86	2.870
Mouth-organs....	6.510	.399	93.87	6.13	12.18	3.052
Umbrella........	2.906	.153	94.74	5.26	3.64	2.379
Velar lobes......	.644	.040	93.79	6.21	1.96	4.900
	10.060	.592	94.11	5.89	17.78	3.003
Mouth-organs....	13.116	.795	93.94	6.06	18.90	2.390
Umbrella........	6.283	.329	94.76	5.24	5.74	1.745
Velar lobes......	1.131	.070	93.81	6.19	2.52	3.600
	20.530	1.194	94.18	5.82	27.16	2.275

PERCENTAGE OF WATER.

It will be seen that the percentage of water in these three different parts of the body is nearly the same. The water-content of the umbrella is, however, constantly higher by about 0.7 per cent as contrasted with the two remaining parts. This is interpreted as probably due to the inclusion in the umbrella of the contents of the stomach, which are mainly liquid. The fact that the velar lobes (which have a large proportion of cellular elements) and the mouth-organs (which have a large proportion of reserve jelly-like substance) are practically identical in water-content shows not only the same degree of water inhibition in both cells and reserve substance, but also an equal distribution of salts.

PERCENTAGE OF NITROGEN IN THE SOLIDS.

Despite the fact of the uniform distribution of water and probably of salts in the body, the nitrogen-contents of these three parts are different. Table 3 shows that the nitrogen-content is highest in the velar lobes, where the cellular elements are most abundant, while it decreases in the mouth-organs and umbrella in the order named. The higher nitrogen-content in the mouth-organs, as compared with the umbrella, is assumed to be due to the relatively greater abundance of the cellular elements in the former. We can infer from these data that the ground substance, or the reserve jelly-like substance, must be composed of organic materials poor in nitrogen. I shall discuss this point later in connection with the probable chemical nature of the reserve material.

The observations made on the normal cassiopea may be summarized as follows:

(1) Relative weights of mouth-organs, umbrella, and velar lobes differ somewhat according to the size of the entire body.

(2) The water-content of the entire body, as well as of different parts, is practically identical throughout the animal's life cycle, so far as followed.

(3) The percentage of nitrogen in the solids is highest in the smallest medusa and the values decrease progressively with increasing body-weight. The percentage of nitrogen is highest in the velar lobes and decreases in the mouth-organs and umbrella in the order named. All these observations are interpreted as indicating that in the structures in which the cellular elements are abundant the nitrogen-content tends to be high.

(4) The ash-content, like the water-content, is probably the same not only in the cassiopea as a whole, but also in each of the three parts throughout the life cycle.

CHEMICAL NATURE OF THE GELATINOUS SUBSTANCE OF CASSIOPEA.

The chemical nature of the jelly-like substance (reserve substance) of various forms of medusæ has been examined by several investigators, but so far as I am aware nothing has been definitely established as to the chemical nature of this puzzling structure.

Krukenberg (1882) considers this substance to be composed of protein material which is easily digested with either pepsin or trypsin and splits off leucin, tyrosin, and other substances not identified. Very recently Macallum (1903) published his extensive investigations on the inorganic constituents of the medusæ *Aurelia* and *Cyanea*, and mentions in the text that "the jelly is constituted of an almost infinitesimally minute network formed of a proteid." In another place Macallum states that "it must be noted that the total amount of proteid in *Aurelia* is very small, ranging between one-seventh and one-eighth of 1 per cent of the total weight of the organism," but in no place in the paper does Macallum mention either the kind of protein present or give any analytical data to show how he determined the quantity of this protein. There are also some chemical observations of a qualitative nature made by Schulze in 1856 and by Schlossberger in 1856. Vernon (1895), who made extensive observations on the phenomena of respiratory exchange in various marine invertebrates, thinks that "the solid organic constituents may not consist by any means of pure proteid."

From the above we see that the true nature of the jelly or ground substance of the medusæ is not determined; this makes it difficult to interpret the various changes noted in the present experiments. It is at once evident, however, that the solids can not be constituted as a whole by the ordinary protein, since the latter usually yields nitrogen to the extent of 12 to 18 per cent, contrasted with 3 per cent in the dried *Cassiopea.*

Even if we calculate the nitrogen in ash-free solids, the value rises no higher than 6 per cent; but this nitrogen value (about 6 per cent) is interesting in view of the fact that it is the value found by Schlossberger (1856) for the chitinous substance. Although the nitrogen-content in the solids alone is not adequate to show that we are dealing with a chitin-like substance, yet recent investigations by Henze (1908) on the identity of the framework of siphonophoræ with chitin and, further, the wide occurrence of chitin in numerous marine invertebrates as shown by Irwin (see Armstrong, 1912) indicate that the ground-substance of the medusæ might be similar. If this supposition turns out to be true, its biological significance becomes at once evident, since chitin is closely related to mucin, which in turn is closely related to the cartilage of the vertebrate skeleton.

EXPERIMENTS ON STARVATION.

LOSS OF WEIGHT IN STARVING CASSIOPEA.

Eight freshly caught normal cassiopeas having different body-weights were subjected to starvation by placing them in filtered sea-water. The filtration was effected with all the precautions adopted by Mayer (1914) and the water (4,500 c.c.) in the vessel was changed once every day. The period of starvation was 21 days and the body-weights were taken every day for the first 6 days and then at 10 and 21 days. The loss of weight is shown in table 4.

TABLE 4.—*Loss of weight in starving Cassiopeas.*

Serial No.	Body-weight of specimen.								
	June 28, 0 day.	June 29, 1 day.	June 30, 2 days.	July 1 3 days.	July 2, 4 days.	July 3, 5 days.	July 4, 6 days.	July 8, 10 days.	July 19, 21 days.
	grams.	*grams.*	*grams.*	*grams.*	*grams.*	*grams.*	*grams.*	*grams.*	*grams.*
3............	164.15	141.48	131.20	123.32	117.19	113.80	104.48	99.34	62.50
2............	156.01	133.42	123.25	115.91	109.43	102.41	95.10	90.40	53.39
1............	152.09	132.05	120.42	110.64	100.29	95.00	88.24	83.32	47.80
Average ..	157.42	135.65	124.95	116.62	108.97	103.74	95.94	91.02	54.56
Per cent..	100.00	86.17	79.38	74.08	69.22	65.90	60.95	57.82	34.66
4............	97.37	85.53	79.38	71.59	68.80	64.27	57.45	48.74	26.35
5............	71.50	64.23	61.78	58.76	54.22	49.97	44.20	36.20	17.51
7............	58.89	51.42	49.57	47.01	44.53	40.85	36.09	29.17	16.53
Average ..	75.92	67.06	63.57	59.12	55.85	51.70	45.91	38.04	20.13
Per cent..	100.00	88.33	83.74	77.87	73.57	68.10	60.47	50.10	26.52
6............	41.08	35.54	33.46	30.72	28.99	27.01	24.58	18.73	9.22
8............	29.46	24.99	23.75	21.94	18.47	15.98	15.41	13.01	5.83
Average ..	35.27	30.26	28.61	26.33	23.73	21.49	20.00	15.86	7.52
Per cent..	100.00	86.08	81.12	74.65	67.28	60.93	56.71	44.97	21.33
Total average..	96.32	83.58	77.85	72.49	67.74	63.66	58.19	52.36	29.97
Per cent......	100.00	86.77	80.83	75.26	70.34	66.19	60.42	54.36	31.11

These eight cassiopeas were divided into three groups composed of the three largest, the three medium, and the two smallest individuals respectively. The object of such grouping was to see whether or not the loss differed according to the size of the animal. Table 4 shows a distinct difference in the loss according to the initial body-weight. In general, the smaller cassiopeas lost relatively much more than did the larger (see table 5). This result agrees with the results found for the starving vertebrates. It is remarkable, however, that cassiopeas after losing nearly 62 to 80 per cent of their initial body-weight appear in no respect different from the normal cassiopea of similar body-weight. It is very difficult to distinguish the starved from the normal by merely looking at them unless the velar lobes happen to be turned upward—as occurs in some of the cassiopeas after several days of starvation.

Mayer (1914) found a simple law which governs the loss of weight in starving cassiopea. The law is represented by the following formula

$$Y = W(1-a)^x$$

where Y represents body-weight W after x days of starvation, and a stands for the constant that gives a fair measure of the rate of loss of weight. Mayer calls this constant a the coefficient of negative metabolism, since a increases as the loss of weight increases.

Extensive data given by Mayer show that this formula accords satisfactorily with his observed data. The starved cassiopeas in my hands showed, however, a sharp drop in the body-weight within 24 hours after commencing starvation; thus the succeeding body-weights were altogether too low as compared with the calculated values, when this formula was applied. Assuming that the initial sudden loss in weight was due to some peculiar conditions not understood at present (possibly the escape of algæ, etc.), I have taken the body-weight, 83.58 grams (see table 4), one day after starvation, as the starting-point, and computed the successive losses of weight according to the formula[1]

$$Y = 83.58\ (1-0.05)^{x-1}$$

Table 5 shows the observed losses of body-weights (from table 4) compared with those calculated by the revised formula.

TABLE 5.

Days.	Observed body-weight.	Calculated body-weight.	Days.	Observed body-weight.	Calculated body-weight.
	grams.	*grams.*		*grams.*	*grams.*
1	83.58	83.58	5	63.66	68.08
2	77.85	79.40	6	58.19	64.68
3	72.49	75.43	10	52.36	52.68
4	67.74	71.66	21	29.97	29.97

By this modification the agreement between the observed and calculated values becomes much more satisfactory. The somewhat lower body-weight obtained in the present experiment as compared with that calculated was probably due to the frequent handling of the animal in order to determine the body-weight daily. The removal of the adherent moisture with filter paper previous to weighing is certainly severe treatment for the delicate medusan body. It is therefore rather a surprise to find that the formula, which has no additional provision for the various experimental conditions except the two factors (number of days and initial body-weights), can represent so closely the data here obtained.

---

[1] The discrepancy between my formula and that of Mayer is due to the fact that Mayer did not commence his observations until after the cassiopeas had remained in aquaria in the laboratory for one day (24 hours), and thus until after all undigested food had been discharged from the medusa. The considerable loss during the first day is largely due to this fact, and also to the loss of much slime soon after being captured.

ANALYSIS OF STARVED CASSIOPEA.

Soon after the final body-weights were determined the specimens were dried in an oven for three days in order to drive out most of the moisture, and then were brought back to the Wistar Institute, where the final drying process was completed two weeks later at 95° to 97° C. The analysis was then made and the results are shown in table 6.

TABLE 6.—*Loss in body-weight and relations of water, solids, and nitrogen in the starved Cassiopeas.*

Serial No.	Initial body-weight.	Final body-weight.	Loss.	Weight of dry solids.	Water.	Solids.	Total nitrogen.	Nitrogen in solids.
	grams.	*grams.*	*p. ct.*		*p. ct.*	*p. ct.*	*mgm.*	*p. ct.*
8	29.455	5.829	80.21	0.305	94.77	5.23	11.06	3.626
6	41.081	9.217	77.56	.467	94.93	5.07	13.58	2.903
7	58.885	16.534	71.92	.847	94.88	5.12	28.56	3.372
5	71.500	17.507	75.52	.970	94.46	5.54	.....	.....
4	97.372	26.351	72.94	1.352	94.87	5.13	39.62	2.930
1	152.092	47.798	68.57	2.391	94.99	5.01	61.60	2.576
2	156.010	53.991	65.39	2.630	95.13	4.87	61.88	2.353
3	164.145	62.495	61.92	3.107	95.03	4.97	82.46	2.654
	[1]96.317	[1]29.965	[1]71.75	.....	[1]94.88	....	.....	.....

[1] Averaged values.

PERCENTAGE OF WATER.

The percentage of water is practically identical in the starved cassiopeas of all sizes, and the average value is 94.88 per cent, which is slightly higher (0.73 per cent) than the average values obtained from the normal cassiopea. A part of this difference may be explained by the fact of the more complete drying of the starved medusa under the favorable conditions of the Institute laboratory as compared with the difficult conditions at the Tortugas. However, as the data given by Mayer also show a slight difference in the water-content between the normal (95.1 per cent) and starved (95.3 per cent), it seems reasonable to conclude that starvation tends to give a slightly higher percentage of water.

PERCENTAGE OF NITROGEN IN THE SOLIDS.

As in the case of the normal cassiopea, the nitrogen-content is highest in the smaller animals and tends to decrease with increasing body-weight. The value of the nitrogen is, however, much higher in the starved cassiopea than in the normal cassiopea of similar body-weight (see table 7).

Thus, 5.56 grams of the normal series gives 2.98 per cent of nitrogen contrasted with 3.63 per cent of nitrogen in the starved cassiopea having a body-weight of 5.83 grams. Such a relation is clearly shown in the majority of cases. It is highly interesting to observe that the percentage of nitrogen is notably high for the final starved body-weight when compared with that in the control with a similar body-weight (table 7), but it is much lower for the initial unstarved body-weight, as will be seen from the extremely low percentage of nitrogen when calculation was made in respect to the initial body-weight instead of the final (see table 7, sixth column). This indicates clearly that during starvation the animal has utilized a considerable amount of the nitrogenous substances—a conclusion which is important, as it answers the main question before us and brings *Cassiopea* in line with the vertebrates in its reaction to starvation conditions. It is highly probable that some fraction of the nitrogen utilized may represent that of the cellular substance. Mayer (1914) noted, from the sections made on the starved cassiopea, not only reduced cell size, but also many degenerated cell-bodies, and there was evidence also of a complete disappearance of some of the cellular elements. Mayer found also a vacuolated condition of the gelatinous substance accompanied by a greater loss of the muscular tonus. The chemical alterations noted above accord with his findings.

TABLE 7.—*Percentage of nitrogen in the normal Cassiopea compared with that in the starved Cassiopea.*

Normal.		Starved.				REMARKS.
Body-weight.	Nitrogen in solids.	Nitrogen in solids.	Body-weight, final.	Body-weight, initial.	Nitrogen in solids.	
grams.	*p. ct.*	*p. ct.*	*grams.*	*grams.*	*p. ct.*	In calculating the percentage of nitrogen in columns (2), (3), (6), the solids were estimated by assuming that the 94.16 per cent of fresh body-weight is represented by the water. The total nitrogen in milligrams found in the solids of the starved cassiopeas was compared with the solids thus obtained at the beginning of the starvation experiment and the percentage values were then computed.
5.56	2.98	3.63	5.83	29.46	0.64	
10.06	3.00	2.90	9.22	41.08	.56	
[1]15.73	2.53	3.37	16.53	58.89	.82	
[2]109.35	2.36	2.65	62.50	164.15	.86	
(1)	(2)	(3)	(4)	(5)	(6)	

[1] Average of two cassiopeas. [2] Data given by Mayer (1914).

Mayer concluded that the percentage of nitrogen in the solids is independent of the period of starvation and is practically identical with that obtained from the non-starved cassiopea. I have, however, found, as stated above, that starvation tends to increase not only the percentage of nitrogen in the solids, but also that the absolute amount of nitrogen shows an increase when the starved cassiopeas were compared with the normals having the same body-weight. The discrepancy between the conclusions drawn by Mayer and by myself is, I believe, due to the fact that Mayer's observations were limited to the larger cassiopeas (body-weights over 100 grams) in which the percentage of nitrogen in the solids shows little variation following the large variations of the body-weight, while the variations in the nitrogen are quite noticeable in the cassiopeas of smaller size. I may add here that the data given by Mayer[1] show also a slight indication of a difference in the nitrogen-content between the normal and starved cassiopeas; thus the nitrogen-content in the normal is 2.35 per cent for a body-weight of 109.3 grams, while the starved gives 2.47 per cent of nitrogen for a final body-weight of 92.4 grams. These differences agree with my own findings.

TABLE 8.—*Data on the content of water, solids, and nitrogen in three parts of starved Cassiopeas.*

Serial No.	Parts.	Weight, fresh.	Weight, dry.	Water.	Solids.	Total nitrogen.	Nitrogen in solids.
		grams.	*grams.*	*p. ct.*	*p. ct.*	*mgm.*	*p. ct.*
8	Mouth-organs.....	2.847	0.154	94.59	5.41	5.60	3.636
	Umbrella..........	2.320	.118	94.91	5.09	3.64	3.085
	Velar lobes.........	.662	.033	95.16	4.84	1.82	5.515
	Total.......	5.829	.305	94.77	5.23	11.06	3.626
6	Mouth-organs.....	5.249	.269	94.88	5.12	7.98	2.966
	Umbrella..........	3.296	.160	95.15	4.85	3.78	2.300
	Velar lobes.........	.672	.038	94.35	5.65	1.82	4.789
	Total.......	9.217	.467	94.93	5.07	13.58	2.903
7	Mouth-organs.....	9.552	.509	94.67	5.33	18.48	3.631
	Umbrella..........	5.561	.262	95.29	4.71	6.72	2.565
	Velar lobes.........	1.421	.076	94.65	5.35	3.36	4.421
	Total.......	16.534	.847	94.88	5.12	28.56	3.372
4	Mouth-organs.....	16.122	.825	94.88	5.12	23.66	2.868
	Umbrella..........	8.193	.412	94.97	5.03	10.92	2.650
	Velar lobes.........	2.036	.115	94.35	5.65	5.04	4.382
	Total.......	26.351	1.352	94.87	5.13	39.62	2.930
1	Mouth-organs.....	26.669	1.335	94.99	5.01	35.42	2.653
	Umbrella..........	19.420	.954	95.09	4.91	21.00	2.201
	Velar lobes.........	1.709	.102	94.03	5.97	5.18	5.078
	Total.......	47.798	2.391	94.99	5.01	61.60	2.576
2	Mouth-organs.....	31.398	1.529	95.13	4.87	36.82	2.408
	Umbrella..........	18.683	.891	95.23	4.77	17.92	2.011
	Velar lobes.........	3.910	.210	94.63	5.37	7.14	3.400
	Total.......	53.991	2.630	95.13	4.87	61.88	2.353
3	Mouth-organs.....	36.214	1.813	94.99	5.01	49.42	2.725
	Umbrella..........	21.796	1.041	95.22	4.78	22.82	2.192
	Velar lobes.........	4.485	.253	94.36	5.64	10.22	4.040
	Total.......	62.495	3.107	95.03	4.97	82.46	2.654
5	Mouth-organs.....	9.212	.506	94.51	5.49	.....	.....
	Umbrella..........	7.297	.367	94.98	5.02	.....	.....
	Velar lobes.........	.998	.061	93.89	6.11	.....	.....
	Total.......	17.507	.934	94.46	5.54	.....	.....

---

[1] Mayer, A. G. 1914. The law governing the loss of weight in starving *Cassiopea*. Carnegie Inst. Wash. Pub. No. 183, table 2, p. 59.

I am thus inclined to believe that even these larger cassiopeas may show a significant degree of alteration after a longer period of starvation than that given them by Mayer, and when the animals have lost 80 per cent or so of their initial body-weight.

ANALYSIS OF THE DIVISIONS OF THE STARVED CASSIOPEA.

Various determinations made on the different parts of the body show that the water-content is practically identical in all the starved cassiopeas. Here also, as in the case of the normal animal, the umbrella gives a slightly higher water value, owing possibly to the inclusion of the stomach contents (water). The percentage of nitrogen in the solids also shows relations similar to those in the normal cassiopea; that is, the nitrogen-content is highest in the velar lobes and lowest in the umbrella, while that for the mouth-organs occupies an intermediate position.

I have shown (in table 6) the loss of weight in body as the result of 21 days of starvation. We noted that in this series the cassiopeas lost on the average as much as 72 per cent of their initial weight, but as to whether they lost in weight proportionally in the different divisions of the body, or whether the loss was otherwise distributed in the three parts, I have found the following relations:

For a final starved body-weight of 29.97 grams (table 6) the relative weights of the mouth-organs, umbrella, and velum are 18, 10, and 2 grams, respectively, when read from figure 1, while the corresponding observed weights were 17.16, 10.82, and 1.99 grams. Reducing these relative weights to ratios we obtain for the body-weight of 29.97 grams

From figure 1, 9:5:1 From observations, 8.6:5.4:1

For the initial body-weight of 96.32 grams (table 6) of the animals subsequently starved to the weight just noted the ratios (from fig. 1) are 8:5:1. Considering the conditions under which the comparison of these data are made, these three ratios may be regarded as equivalent and show that the three parts of the body have, as it were, reversed the process of growth during starvation. The strictly proportional loss in these three parts accounts for the normal appearance of the animals, even after they have lost as much as 62 to 80 per cent of their original weight.

The statement made in my preliminary report (1916) that "in the starved cassiopeæ the relative weights of the three parts coincide with those given by the normal cassiopea, which have a body-weight similar to the starved cassiopea at the end of the test, and not to the relative weights at the beginning of the experiment," should therefore be changed to a more general statement by omitting "and not to the relative weights at the beginning of the experiment," since the deviations of the proportional values after starvation are so slight, whether these are compared with the proportions given by the normal examples having either the final or initial body-weights. More exact statements can be made after we have obtained a large number of data on both the normal and starved specimens.

Briefly summarized, the results of the observations made on the starved cassiopea are as follows:

(1) In general the smaller cassiopea loses relatively more in weight than does the larger cassiopea.

(2) The percentage of water found in the entire body, as well as in the three different parts, is nearly the same in all sizes of cassiopea. However, the values of the water-content in the starved appear to be slightly higher than that found in the normal cassiopea.

(3) The nitrogen-content of the entire body is higher in the small than in the larger cassiopea, as in the case of the normal animals.

(4) However, the absolute amount of nitrogen found in the starved cassiopea is considerably higher than in the normal having the same body-weight. It was noted also that, although high when compared with a normal specimen equal in weight to the starved animal, it is very low for the initial body-weight of the starved animal. This shows that the nitrogen has also been consumed during the period of starvation.

(5) The nitrogen-contents for the different parts of the body are similar in their relations to those found in the normal cassiopea.

(6) The loss in weight of the different parts is of such a character that their proportion in the starved remain similar to those in the normal cassiopea.

LITERATURE CITED.

ARMSTRONG, E. FRANKLAND. 1912. The simple carbohydrates and glucosides. Monographs on biochemistry. Longmans, Green and Co.

HATAI, S. 1916. Changes in the chemical compositon of starving Cassiopea, *Cassiopea xamachana*. Carnegie Inst. Wash. Year Book No. 15, pp. 206–207.

———. 1917. Changes in the composition of the entire body of the albino rat during the life span. Amer. Jour. Anat., vol. 21, No. 1.

HENZE, M. 1908. Notiz ueber die chem. Zusammens. der Gerustsubstanz von *Velella spirans*. Zeitschr. f. physiol. Chem., Bd. 55.

KRUKENBERG, C. FR. W. 1882. Das organische Substrat des Medusenkörpers. Vergleichend-physiologische Studien. 2 Reihe. Heidelberg.

MACALLUM, A. B. 1903. On the inorganic composition of the medusa *Aurelia flavidula* and *Cyanea arctica*. Jour. Physiol., vol. 29.

MAYER, A. G. 1914. The law governing the loss of weight in starving *Cassiopea*. Carnegie Inst. Wash. Pub. No. 183, pp. 55–82.

SCHLOSSBERGER, J. E. 1856. Allgemeinen und vergleichenden Thier-chemie. Leipzig u. Heidelberg.

SCHULZE, E. 1856. Ueber den Bau der Gallertscheibe der Medusen. Müller's Archiv, vol. 311.

VERNON, H. M. 1895. The respiratory exchange of the lower marine invertebrates. Jour. Physiol., vol. 19.

VI.

THE HABITS AND REACTIONS OF A COMATULID, TROPIOMETRA CARINATA.

By HUBERT LYMAN CLARK,
Curator of Echinoderms, Museum of Comparative Zoölogy, Cambridge, Massachusetts.

THE HABITS AND REACTIONS OF A COMATULID, TROPIOMETRA CARINATA.

By Hubert Lyman Clark.

INTRODUCTION.

One of the most interesting echinoderms taken during the recent visit of the Carnegie Institution's party to Tobago (March and April 1916) was the comatulid *Tropiometra carinata* (Lamarck). This crinoid was common in Buccoo Bay, and while Dr. Mortensen successfully undertook the study of its development, I made such observations on its habits and reactions as time and circumstances permitted. The habitat of most of the individuals noted was so completely different from any in which we had previously seen comatulids that we naturally inferred the reactions would differ markedly from those of the species studied in Torres Strait.[1] I therefore performed a few simple experiments, the results of which are here recorded, together with such observations as were made on the animal in its natural environment.

SYSTEMATIC POSITION.

Comparison of the comatulids taken at Tobago with a considerable series of specimens from several stations on the Brazilian coast, south of the Amazon, shows that they are unquestionably identical. For this Brazilian species, Mr. A. H. Clark has revived an old name of Gay's, *picta*, regarding it as "a perfectly good species," "most obviously differing from *carinata* in the greater length of the outer cirrus segments" (Proc. U. S. Nat. Mus. 1911, vol. 40, pp. 35, 36). On comparing the Brazilian and Tobagoan material with specimens from Mauritius and Zanzibar, I was utterly unable to detect any differences, either in the cirri or in any other characters. On writing to Mr. Clark of my difficulties, he very kindly replied that while the species of *Tropiometra* are very difficult to distinguish, he finds "no difficulty in distinguishing" the group in which he places *picta* from that in which he places *carinata*, "by the difference in length of the outer cirrus segments." On receipt of his letter, I went over the cirri again, but I found myself absolutely unable to detect the difference named. I have therefore very reluctantly reached the conclusion that I can not recognize *picta* as a valid species, but must designate my Tobagoan comatulids by their old Lamarckian name.

---

[1] Comatulids of Torres Strait, H. L. Clark, Carnegie Inst. Wash. Pub. No. 212, pp. 95–125, 1915.

HABITAT.

We first found *Tropiometra* in Buccoo Bay, where it occurs in water from a few inches to several feet in depth at low tide; indeed, at the lowest tides some individuals are probably out of the water, in part at least, for a short time. The bottom which they frequent is made up largely of *Porites* fragments, usually more or less covered by a growth of *Corallina* and *Halimeda*. Scattered over it there is also a sparse growth of short eelgrass (*Zostera*). As a rule the comatulids hold themselves in an erect position by means of their stout cirri, which are customarily grasping a bit of *Porites*. Sometimes the body is more or less completely shaded by a clump of eelgrass or seaweed, but this is not usually the case. Generally the individuals are solitary, but occasionally five or six may be found about a single clump of *Porites* or of eelgrass. They are not really abundant, but twenty or more may be gathered in half an hour or less. Now and then we found individuals living underneath slabs and large fragments of coral; this was particularly true on Buccoo Reef, where, later, a number of specimens were discovered. All were in shaded places, suspended arms down, and not in the usual erect position.

COLOR AND SIZE.

The first specimens of *Tropiometra* seen appeared to be uniformly dark purple, purplish-brown, reddish-brown, or bright brown, but a closer examination showed that uniform coloring was very rare. Almost always a longitudinal dorsal stripe on each arm, or transverse bands of more or less width, or both, are present. The color of these markings is yellow of some shade, often dull and buffy, but not rarely quite bright. In some individuals the pinnules are also cross-banded with yellow, and the distal pinnules may be uniformly brownish yellow. The cirri are yellow brown, at least dorsally, but are often more or less dusky or purplish on the oral surface; in nearly all individuals, however, both young and old, the terminal two or three segments (except the claw) have a dusky spot on the oral side. This marking seems to be a very constant character in Tobagoan specimens. Occasional individuals are found in which the pinnules and dorsal side of the arms are plenteously besprinkled with silvery-white, giving them an exceptionally handsome appearance.

All of the small specimens found were brownish-yellow or bright brown, more or less marked and banded with purple, and this general coloration is not rare in adults, particularly in those found under slabs of rock on Buccoo Reef and in similar shaded places. Some of these individuals were very handsome in their brilliant array of purple and gold, and it was hard to believe they were really identical with the dull-colored specimens from the shallows of Buccoo Bay. A natural inference from the specimens seen is that the young are uniformly yellow[1] or brownish-yellow, and that the purple pigment develops as they mature, in some individuals completely obliterating the original color, but usually appearing simply as spots, blotches, and cross-bands. One could scarcely avoid the impression that the development of the pigment is associated with life in the open sunlight, but there was no chance to secure an answer to the interesting question which suggests itself: Do the bright-colored individuals avoid the sun because they lack pigment or do they lack pigment because they have never lived exposed to the sun?

Several very small individuals were found under rocks on Buccoo Reef, but the smallest one seen (having arms about 18 mm. long) was discovered in a clump of *Corallina.* The largest specimen measured had an arm-length of nearly 100 mm., and a few specimens exceeded that, but the great majority had arms 60 to 80 mm. long. No specimens with more or fewer than ten arms were noted, in more than 200 examined, but several cases of arms forked distally were seen and in one case a forked pinnule was noted. As a rule the arms were approximately equal, but in some individuals those of one side were distinctly shorter than the others. In such cases, however, it was usually obvious that the short arms were regenerating.

FOOD.

Although some individuals live suspended under rocks with the mouth down, while the great majority are erect with the opening upwards, the character of the food is in all cases planktonic—that is, it does not consist of such organic matter as happens to fall on the disk and arms, but is made up of the living, active plankton. When examined under the microscope, the stomach contents are seen to consist of a mixture of vegetable and animal food, the former predominating.

The plants are diatoms and unicellular green algæ, with occasional fragments of other seaweeds. The condition of the green algæ showed that most, if not all, were ingested while living, and the same seemed to be true of the diatoms. Of animals, crustacea were most frequently noted, but a few foraminifera were also seen. The crustaceans were minute amphipods, copepods, and crab zoœas, and all were in such condition as to leave no doubt that they were alive when ingested. Just how such active animals are actually captured and forced into the mouth is not clear. As compared with the comatulids studied in Torres Strait, *Tropiometra* shows an unusual proportion of animal food in its diet, but this may of course be purely a local or seasonal matter.

---

[1] In support of A. H. Clark's views on the color of young crinoids. See Geog. Jour. London, Dec. 1908, p. 606.

LOCOMOTION.

Like most of the comatulids hitherto studied, *Tropiometra* is exceedingly sluggish. So far as my observations go, it never swims. Dropped or suspended in deep water, it makes only the most feeble arm-movements and, unless artificially prevented, it sinks rapidly to the bottom. When, as a result of some adequate stimulus, *Tropiometra* does move, the movement is similar to that of *Comanthus*. That is, it is accomplished solely by means of the arms, and no particular arms take precedence in the matter. In one respect the attitude differs from that of *Comanthus*, for the body is much more distinctly raised. This is obviously to keep the cirri wholly above the substratum and thus prevent their accidentally catching on it. The movement is always slow and labored and does not continue long. When overturned so that the cirri are up, a normal healthy *Tropiometra* invariably rights itself at once, sometimes only very slowly and deliberately, but usually without delay. The process is, as in other comatulids, by several arms raising up their side of the body until one or more of them or some of the neighboring arms can reach over backwards and secure a hold which will enable them to pull the body over. The cirri play no part whatever in this process, any more than in actual locomotion; they are very inert, move slowly and slightly, and are used only as organs of attachment, a purpose which they serve admirably. The pinnules, even near the tip of the arm, have only weakly hooked tips and are but slightly viscid, and it is consequently difficult for *Tropiometra* to move or even to right itself except on a rough and rather firm surface. No individuals were seen moving about under normal conditions, but when placed on a bare, sandy bottom they crept steadily to its margin of coralline algæ. Many specimens were planted on the reef-flat near the laboratory and most of these ultimately disappeared, but whether they crept away or were carried off by tidal movements it is impossible to say.

REACTIONS TO LIGHT.

The remarkable habitat of *Tropiometra* in Buccoo Bay naturally suggested that in its reactions to light it would be positively phototactic. A tank 6 feet long, a foot wide, and 8 inches deep, having the bottom covered with broken coral and stones, was used for the experiments. It was placed near high-water mark on the beach, in the sun, and two boards, 5 or 6 inches wide, were laid across it, giving shaded areas; one end was made quite dark by covering with a broad piece of board and burying it in the sand. Numerous individuals of *Tropiometra* were tested in this tank on many different occasions and under diverse conditions of temperature. The results were surprisingly unsatisfactory. No individual showed any decided reaction to light and none sought the darkness. In most cases, when the specimen was fresh from Buccoo Bay, if placed in a shaded area it remained there; if placed in the sun close to a shaded area, it moved into the shade, but if the board were then removed, it made no effort to move out of the sun; if placed in the sun some inches from shade, it remained quietly in the light. Generally the individuals were placed upside down in the tank, so that the process of righting might serve to initiate movement. But although they righted themselves promptly, they rarely wandered more than a few inches thereafter. If placed in the tank right side up, they were usually content to remain where put, regardless of whether it was in sun or shade.

Experiments were made particularly to see whether the brightly colored specimens from under rocks reacted differently from the dark individuals from Buccoo Bay, but no constant differences could be detected. Brightly colored examples of *Tropiometra* seemed to be a little less hardy and became sluggish and inert in the tank more quickly than the dark ones, but the difference was not striking. The final conclusion reached, as a result of all the experiments and observations, is that *Tropiometra* at Tobago is *slightly negatively phototactic*, but that other factors in the environment in Buccoo Bay neutralize this tendency almost or quite completely, while on Buccoo Reef it is for some reason strengthened.

REACTIONS TO TEMPERATURE.

While it was not feasible to produce really low temperatures, water in the tank fell to 22° C. during the night. At this temperature *Tropiometra* showed no signs of discomfort or stimulation. When the water was gradually raised to 33° or 34°, responses to mechanical stimulation were normal. At 35.5° two of the four specimens tested were quite unresponsive, but the other two gave normal responses by arm-movements. One continued to respond at 36.5°, but at 37.5° was inert. After a few moments at that temperature the four specimens were placed in normal sea-water at 28°, but none recovered. Individuals taken from water at 28° and placed in that at 33° continued to give responses with the arms. At 34°, however, the arms were quiescent, but the pinnules responded. Complete recovery of these specimens resulted when after a few moments they were again placed in water at 28°. Individuals placed in water at 36° gave no responses and failed to recover; they became quite rigid. These experiments show that as compared with the comatulids of Torres Strait, *Tropiometra* has a surprisingly large temperature range. This is quite in keeping with their habitat in Buccoo Bay, for while the water there probably never falls below 22° C., there is no doubt that at the lowest tides it rises in the shallows to 32° and possibly to 34°. Even should it on rare occasions exceed this figure for a short time, it is evident that *Tropiometra* would easily survive. Owing to the comatulid's inertness it was impossible to determine the optimum temperature. In water of 33° reactions were not consistently different from what they were at 25°; nevertheless individuals in water at 33° assumed a somewhat wilted appearance after a time, which was not the case in water at 28° and lower. Healthy individuals gave no response to a sudden change of 2°, but there was generally an evident reaction to an abrupt change of 5°.

REACTIONS TO SALINITY.

Experiments to determine whether *Tropiometra* is sensitive to changes in the salinity of the water in which it lives gave very interesting results. Two individuals were placed in an aquarium containing 5 liters of ordinary sea-water and two specimens of similar size and appearance were placed in another aquarium containing 5 liters of 90 per cent sea-water (*i. e.*, 4.5 liters of sea-water plus 500 c.c. of rain-water). After 12 hours all were normal and one could not tell from the responses to mechanical stimuli which were in the diluted sea-water. Similar experiments were tried with water only 80 per cent and 75 per cent sea-water. Such water extracted color from the comatulids and after 12 hours was distinctly yellow, yet the tropiometras survived and responded to the transfer to normal sea-water by distinct arm-movements.

Experiments were then made to see how concentrated a sea-water could be survived. Two specimens, one dark-colored and one yellow and purple, survived with no apparent injury 12 hours in only 2 liters of water 10 per cent more saline than normal. The brightly colored one was more active after the experiment than the other, which was not what I had expected in view of the greater evaporation in the natural habitat of the dark specimen. A similar experiment with water 20 per cent more saline than normal was tried. A brightly colored individual was still alive after 3 hours; it was then transferred to normal sea-water and lived over night. A dark-colored specimen was still alive after 4½ hours, but although it was then transferred to normal sea-water, it failed to revive. These experiments show a surprising indifference on the part of *Tropiometra* to the salinity of the water in which it lives.

REACTIONS TO CERTAIN CHEMICALS.

Although no experiments were made to test the reactions to chemicals, it may be worth while to record the observations made in other connections. Indifference to the amount of CO_2 in the water was noticeable, particularly in view of the sensitiveness of Torres Strait comatulids to that substance. As already stated, 2 specimens lived over night in only 2 liters of sea-water, apparently without discomfort, and 4 or 5 specimens were often kept over night in aquaria with not more than 6 or 7 liters. Unlike the comatulids tried in Torres Strait, *Tropiometra* when fresh from the sea responds to a saturated solution of $MgSO_4$ in sea-water, like other echinoderms, the whole muscular system becoming relaxed. The response was much less complete if the individual had been for some time in a limited amount of sea-water; probably the presence of CO_2 made the difference. Response to alcohol and formalin was, as in other comatulids, by a contraction first of the dorsal side of the arms and then of the ventral. Only exceptionally, and then by individuals which had been long in tank or aquaria, were the arms cast off.

SUMMARY.

The common habitat of *Tropiometra* at Tobago, Buccoo Bay, is unusual for a crinoid, the bottom being covered with coralline algæ and eelgrass, the water shallow, and the comatulids exposed to the full glare of the tropical sun.

Reactions to light, temperature, salinity, and CO_2 all show *Tropiometra* to be remarkably insensitive and hardy. It shows only feeble phototactic reactions, has a temperature range of at least 15° C., a salinity range well over 40 per cent, and withstands a considerable but undetermined amount of CO_2 without injury.

VII.

STUDIES ON THE PHYSIOLOGY OF THE NERVOUS SYSTEM OF CASSIOPEA XAMACHANA.

BY LEWIS R. CARY,
Department of Biology, Princeton University.

Eighteen text-figures.

STUDIES ON THE PHYSIOLOGY OF THE NERVOUS SYSTEM OF CASSIOPEA XAMACHANA.

By Lewis R. Cary.

INTRODUCTION.

In this paper are gathered the results of several distinct lines of experimentation, all of which have this in common—that they deal with some phase of the physiology of the nervous system of *Cassiopea* and represent portions of a general program of research on the nervous system of the lower animals. On account of its ability to live under adverse conditions and to withstand practically any type of operation, *Cassiopea* is an especially favorable form for experimentation and has been used as a subject for many researches. On regeneration by Stockard, Zeleny, and Goldfarb; for the study of pulsation, rate of nerve-conduction, effect of starvation, etc., by Mayer; on chemical composition and chemical changes during starvation by Hatai; on reaction to temperature, permeability, and nerve-conduction by Harvey, and the researches herein recorded by the author.

In very few types of animals, where the recognizable activities are entirely under the control of nerve-centers, can all these structures be removed without causing death, or at least serious injuries which will render abnormal practically all physiological activities. Among the Scyphomedusæ, *Cassiopea,* in common with many other Rhizostomæ, is distinguished for the density of its mesoglœa, but it stands preeminent as a laboratory marine animal because of the fact that its normal habitat, being in shallow lagoons of relatively stagnant water, fits it especially for the adverse environment encountered under experimental conditions in laboratory aquaria. The sense-organs (nerve-centers), because of their position around the periphery of the disk, can be removed with very little injury to any of the other tissues of the body.

Since, also, these structures are equally spaced around the circumference of the disk, any portion of the body can, by the appropriate operation, be deprived of its normal nerve-supply, while retaining its other relations undisturbed. Then, too, the rapidity with which the centers are replaced by the regeneration of normally functioning new ones permits experimenting on any given area of tissues, first without and later under the influence of nervous impulses.

In studying regeneration in *Cassiopea* I found that the rhopalia exert a decided influence on the rate of regeneration, which is most marked in the early stages of any experiments, and that this influence was in the major part independent of muscular activity. The observations were, therefore, extended to involve other factors as the basis of comparison, since it seemed evident that the influence on regeneration must be in some manner exerted through fundamental metabolic activity, which might be expected to be measurable on some other basis than the rate of regeneration. These expectations were fulfilled when either the general metabolism, as measured by total CO_2 production, the loss of weight during starvation, or the changes in the rate of nerve-conduction in response to changes in temperature, was used as the standard of comparison.

The same types of operations were used in experiments on regeneration, general metabolism, loss of weight during starvation, and the influence of the sense-organs on the change in rate of nerve-conduction in response to changes in temperature, so that the results obtained by use of these distinct standards of measurement are directly comparable.

The experiments on the influence of the nerve-centers on the loss of weight during starvation, on account of the operations employed, necessarily involved regeneration at the same time, and in some instances this factor was measured as well as that for which the experiments were primarily carried out. Conversely the experiments on regeneration, since only the disks were used, were carried out on starving medusæ and the decrease in both area and weight of the half disks was often recorded for these experiments. While both these factors (*i. e.* regeneration and decrease in size and weight) were involved in the experiments on the rate of nerve-conduction, each experiment extended over so short a period of time that no measurements of the amount of regeneration were possible, and the loss of weight, while actually small, was entirely in accord with the results obtained from the more extended experiments. Thus the high rates of pulsation brought about by nerve-conduction, independent of the rhopalia, tended especially to emphasize the inadequacy of differences in motor activity as the explanation of difference in rate of metabolism as expressed by loss of weight.

TECHNIQUE OF THE EXPERIMENTS AND SOME RESULTS OF OPERATIONS THAT APPLY EQUALLY TO ALL EXPERIMENTS.

The natural habitat of *Cassiopea* is in shallow lagoons, usually in areas where mangroves are abundant, where the water is daily subjected to marked fluctuations in temperature, and where the salinity is most affected by precipitation. In such locations, many of which are practically stagnant, the variations in salinity are of wide range, as evaporation is rapid during the summer months, when also the precipitation is greatest. Such lagoons usually support an abundant algal flora, so that the gaseous content of the water varies considerably and large quantities of organic acids are continually being generated. The environmental conditions consequently vary widely from time to time and are always decidedly different from those in the open tropical ocean. Indeed, so thoroughly inured to this environment is *Cassiopea* that it thrives better under laboratory or lagoon conditions than in pure sea-water.

At Tortugas the proper environment for this form is restricted to the moat, at Fort Jefferson, which is 50 feet wide, extends entirely around the fort, and communicates with the open water outside at only two points through narrow entrances which are entirely cut off at extreme low tide. As the mean rise and fall of tides is in this region only about 1.5 feet, the amount of change in the water of the moat is slight and currents are scarcely detectable at a distance of 200 yards from the entrances. Over the greater portion of the moat, the bottom is densely covered with a mat of filamentous algæ, while its side walls furnish a place of attachment for innumerable specimens of hydroids, bryozoa, tunicates, annelids, mollusks, and several species of corals. Among the algæ the cassiopeas lie on their aboral surfaces, with their branching mouth-arms giving a flower-like appearance and with the bell-margin pulsating slowly. In adult specimens, movement from place to place by their own activity apparently occurs very rarely, if at all. Even young medusæ, not more than 2 cm. in diameter, seldom are raised from the bottom by their own pulsation, and it is doubtful if the larger specimens are capable of moving about by swimming movements of the disk.

The exumbrella surface is depressed in the center and can be used as a sucking disk by which a medusa can attach itself firmly to a vertical surface or resist removal from the bottom. This surface has a simple layer of epithelial cells over the mesoglœa and is not provided with either nerves or muscle-cells.

When resting in its normal position on the bottom, the languid pulsations of the bell-margin create currents sufficient to bring the food material (which consists entirely of minute organisms) onto the mouth-arms, where it is taken in through the numerous small openings into which the original oral cavity has been subdivided by the branching and anastomosing of the oral arms.

When brought into the laboratory the medusæ may be kept in aquaria through which a small stream of water is run slowly—or, if only 3 or 4 specimens are placed in a 10-liter jar, they will remain in normal condition if the water is changed once every 48 hours. The unusual agitation of the water attendant upon changing that in the jars or the handling of the specimens causes a copious secretion of mucus from glands situated on the oral arms, so that the less often they are disturbed the more nearly normal they will remain.

Specimens kept under either of the conditions just mentioned gave the expected results when used for any of the experiments, but in order that any possible source of error from differences of previous treatment might be eliminated, no specimens were used for routine experiments which had been in aquaria more than 12 hours before the time of the first operation.

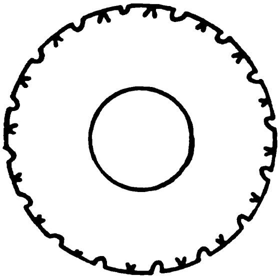
In all experiments which were to extend over more than a few hours, each disk (or its two separated halves) was kept in a jar containing not less than 4 liters of sea-water.

As most of the experiments required the handling of the specimens under observation once each day they were transferred to clean jars of fresh sea-water at that time.

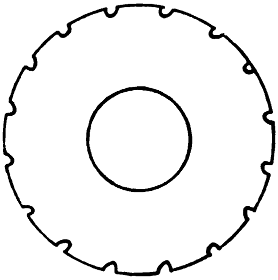
The nervous stimulus causing pulsation originates in the sense-organs, probably through a chemical reaction liberating sodium, at the nerve-centers (Mayer, 1908). At any given time only a single sense-organ, which at that moment is discharging at the highest rate, controls the rate of pulsation. Consequently all the nerve-centers except one may be removed without seriously interfering with the activities of the medusa; when the last one has been removed the specimen will remain quiescent until a new sense-organ has been regenerated with sufficient completeness to take up again the normal chemical reactions which liberate sodium.

Any portion of the disk adjacent to where the nerve-centers have been removed may be insulated from the influence of the remaining centers by destroying the continuity of the subumbrella ectoderm, in which alone the muscles and nervous elements are contained, while still retaining its continuity with the remainder of the disk through the mesoglœa. This mesoglœa is of sufficient thickness to afford a support for isolated areas of the active tissues even when almost the entire ectodermal covering has been removed. Also when the medusæ are kept in normal sea-water it is little subject to bacterial action and can be maintained in an apparently healthy condition even when considerably more than half of the ectodermal layer has been removed.

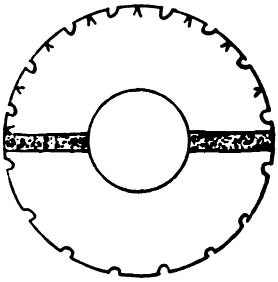
Three comparisons were made in the experiments dealing with regeneration, loss of weight during starvation, total metabolism, and the influence of the sense-organs on the change in rate of pulsation in response to change in temperature. For all these experiments the oral arms and stomachs were removed and the disk alone used, to avoid the contamination of the water by mucus and to facilitate the operations. In each experiment disks of the same size were used, although specimens of the same diameter showed considerable variation in weight due to differences in the thickness of the mesoglœa. For studying the general metabolism large specimens were used because it was easier to perform the necessary operations. In the first type of experiment, normal disks, or half-disks, were compared with others that had been rendered inactive by the removal of all sense-organs (figs. 1, 2, 3). The sense-organs were cut out from the border of the disk by a sharp cork-borer just large enough to completely remove this structure at one stroke. From between the sense-organs of the "active" specimens a piece of tissue of equal size was removed so that the amount of injury was the same for both specimens.



1



2



3

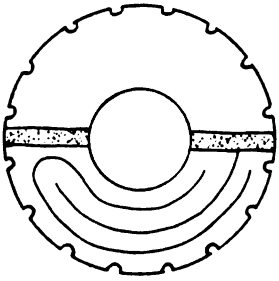
FIGS. 1 and 2.—Entire medusæ. From fig. 2 all the sense-organs have been removed. From fig. 1 an equal amount of tissue has been removed from between the sense-organs.

FIG. 3.—A medusa prepared with active and inactive halves by removing the sense-organs from half of the disk and then insulating this half from that on which the sense-organs remain by removing two diametrically opposite strips of subumbrella tissue, shown as stippled areas. A piece of tissue was removed from between each pair of sense-organs on the active half-disk. The inner circle represents the limits of the cavity in which the amount of regeneration was measured.

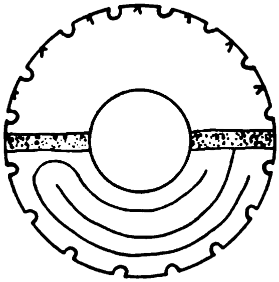
In all the experiments on regeneration recorded in the tables given in this paper the halves of each disk were insulated by the removal of two diametrically opposite strips of subumbrella ectoderm extending from the periphery of the disk on each side to a cavity in the center of the disk where the amount of regeneration was measured (fig. 3). In later regeneration experiments and all other experiments where this type of operation was used the medusa disk was separated into halves and the regular operation was performed upon each half. For measuring the amount of regeneration a disk of tissue 22 mm. in diameter was removed from a similar part of each half-disk. This complete separation of the half-disks did away with the folding of the disks at the point where the insulating strips were removed, which often interfered with accurate measurement of the regenerated tissue. (These series will be referred to as the "active" and "inactive" series.)

In the second type of experiments normal disks or half-disks were compared with others from which all sense-organs had been removed, but in which the subumbrella muscles had been activated by electrical stimulation. In entire half-disks, from which the central portion has been removed, a circuit wave of contraction may be initiated by induction shocks and maintained for as many as 11 days unless interrupted by some strong stimulus (Mayer, *op. cit.*; Harvey, 1911).

When half-disks were employed it was necessary to make two incisions through the muscles and nerves in such a manner that an endless labyrinth of these tissues would be formed (fig. 4), in which the circuit wave of contraction could be maintained.



4



5

FIG. 4.—A medusa prepared for comparison of activated and inactive half-disks. All the sense-organs have been removed from both halves, while in the subumbrella tissues of one of them an endless labyrinth has been formed in which a circuit wave of contraction will be maintained as soon as it has been initiated by induction shocks.

FIG. 5.—A medusa prepared for the comparison of active and activated half-disks.

These series will be referred to as the "active and activated" series.

In the third type of experiments "activated" specimens prepared in the manner just described were compared with "inactive" specimens (fig. 5).

In all instances where active and activated specimens were compared it was observed that the pulsation-rate of the activated specimens was at first about 3 times as great as that of the active specimens. The difference in rate became progressively greater for about the first 24 hours, both on account of an actual increase in that of the activated as well as a decrease in the rate of those with sense-organs intact, until at the end of this time the activated specimen was often pulsating 10 times as rapidly as its mate. If an entire disk or unmutilated half disk were used as the active specimen of any pair its pulsations appeared to be much more vigorous than its mate, in the muscles of which a circuit wave of contraction was maintained, because the bell-margin was folded inward onto the body of the disk at each contraction. When, however, a similar labyrinth had been formed in the subumbrella tissues of the active specimen and the continuity of the sheet of muscles had been destroyed, the bell-margin was no longer folded over and its pulsations lacked the appearance of greater vigor than that shown by the activated specimens.

When simultaneous kymograph records were made of the pulsations of the halves of a disk, one active and the other activated, each with an endless labyrinth formed in its subumbrella tissues, the amplitudes of contraction were found to be equal, showing that the apparent character of the contraction was a function of the normal spatial relationship of the muscle tissue and that when similar series of cuts were made in the active tissues of each half-disk of any pair the rate of pulsation was a true measure of the work done.

Besides a control series carried along as a check for each experiment, a large series of half-disks were prepared, so that each member of any pair (*i. e.*, halves of the same disk) had been subjected to the same operation. Fifty pairs of such half-disks of the three operative types used were recorded for rate of regeneration and loss of weight during starvation, and 10 pairs for total metabolism, with the result that in every instance the similarly prepared halves of each disk gave results which were within the limits of error of the unit of measurements employed in each set of experiments. It is evident from these results that the medusa disks can be safely considered as physiological units which may be subdivided, with the expectation that like areas or masses of tissue of any one disk will respond in an equal degree to experimental changes in environmental conditions.

EXPERIMENTAL.

INFLUENCE OF NERVE-CENTERS ON RATE OF REGENERATION.

Previous studies on the influence of the nervous system upon regeneration have given very divergent results which can hardly be reconciled even when we consider that widely separated groups of animals were used as the material for experimentation.

While certain students of this problem (Herbst, Goldstein, Walter, Wolff) have taken the position that the nervous system in general, or some portion of it (sensory ganglion, Herbst, Walter), exerts a stimulus necessary for the complete regeneration of normal structures, on the contrary other workers have attributed less and less importance to these influences. The intermediate position that the influence of the nervous system is indirect, being exerted mainly through the controlling of motor activity, is well expressed by Child (1905*a*) in the statement concerning anterior regeneration in *Leptoplana*, that, "as in posterior regeneration, there is close parallelism between the rapidity, amount, and completeness of anterior and lateral regeneration and the characteristic motor activity of the part concerned."

Goldfarb (1909) concludes from his experiments on newts, earthworms, and planarians that "these experiments * * * should make one cautious about accepting the view of the direct or even indirect influence of a nervous influence on regeneration."

In all these studies the point at issue has been whether or not complete regeneration of typical structures is possible in the absence of any influences exerted through the central nervous system. An affirmative answer to this question is apparently held, at least by certain of these investigators, to settle finally the question of nervous influence without any consideration being given to the comparison of the course of the regenerative process in animals in which the nervous system was removed from the regenerating area and those in which the nervous system had been uninjured in the portion of the animal left to regenerate. In only relatively few animals can the nerve-centers be removed without bringing about the destruction of, or degenerative changes in, other intimately connected portions of the nervous system, so that this type of operation has not been frequently undertaken.

Zeleny (1907) and Stockard (1908) removed the marginal sense-organs from the disk of *Cassiopea xamachana* to determine the influence of these structures on the rate of regeneration. Both report that there was no evidence of any regulatory influence. In Zeleny's experiments the entire margin of the disk with its sense-organs was removed and the rate of regeneration in these individuals compared with others in which the bell-margin and sense-organs were intact. In Stockard's experiments the results obtained from specimens prepared as above described were supplemented by those obtained with individuals from which one half of the marginal sense-organs was removed, while from the other half an equal amount of tissue was cut from between the sense-organs. The two halves were insulated by the removal of two diametrically opposite strips of subumbrella ectoderm. In both experiments the rate of regeneration was measured inward from the periphery of a cavity in the center of the disk from which a circular piece of tissue had been removed.

In both these researches the experiments were carried out with the view of ascertaining the influence of muscular activity and thus indirectly of the nervous system on the rate of regeneration. In each case it was held that there was no constant difference in the rate of regeneration between the active and inactive individuals.

In the course of my studies, which were taken up primarily to reëxamine the work of Stockard and Zeleny upon this point, I discovered that the marginal sense-organs influence regeneration independently of their control of muscular activity. Such an influence of the sense-organs can be accounted for either on the ground that metabolic activities, not expressed by muscular activity, are under the control of the sense-organs, or that a direct trophic influence is exerted by the sense-organs on the regenerating tissues. A series of determinations with the "biometer" of the rate of CO_2 production by specimens under different experimental conditions, for which I am indebted to Dr. S. Tashiro, shows that the first of the two alternatives just mentioned offers a satisfactory explanation of the observed facts.

The course of normal regeneration as shown when a disk of tissue is removed from the center of a medusa disk is that first the ectodermal epithelium from both the exumbrella and subumbrella surfaces grows inwardly over the exposed surface of the mesoglœa until that extending from the two surfaces has come in contact. Fusion of the two layers takes place at once and then a sheet of new tissue begins to extend across the cavity left by the removal of the disk of tissue. In this sheet of new tissue no mesoglœa layer is at first distinguishable, the two layers of epithelium being in direct contact at their inner surfaces.

Just as in the embryonic development of all Cnidaria, the mesoglœa makes its first appearance as an acellular layer of gelatinous material secreted by the cells of the layers it is to separate. In later stages of regeneration the newly formed mesoglœa fuses insensibly with that of the body of the disk at the periphery of the cavity. When the sheet of regenerated tissue reaches the center of the cavity its edges fuse, but leave for more than 24 hours a recognizable scar at the point where fusion took place. In the specimens used in the following experiments this scar was at first eccentrically placed, but within the first day after it had been formed it came to lie centrally in the sheet of new tissue. From this time the most noticeable change was the increase in the thickness of the regenerated tissue, which within two weeks—in specimens in sea-water—could not be distinguished from the surrounding tissue. Long before this time tissue differentiation had taken place, so that functional nerves, muscles, gland and sensory cells had been formed from the syncitial mass into which the epithelial layers had been converted in the earliest stages of regeneration.

EXPERIMENTS WITH ENTIRE DISKS.

When the rates of regeneration of a series of active and inactive entire disks is compared it is found that in about 75 per cent of all the experiments the regeneration is most rapid in the active specimens. In the remaining disks the amount regenerated at any given time is (in about 10 per cent of the pairs) found to be equal within the limits of accuracy of measurement, while in about 15 per cent of the pairs of disks regeneration was greatest in the inactive specimens.

The results of many different kinds of experiments upon *Cassiopea* show wide variations in the sensitivity and metabolic activity in this animal. It therefore seems evident that the conflicting results obtained from specimens subjected to this type of operation are to be explained as individual variations in physiological activity.

More dependable results may be expected from specimens prepared according to Stockard's method (fig. 3), where individual variations in physiological activity are eliminated.

After this operation the inactive half of each specimen is moved about by the pulsation of the active half, so that there can be little difference in the degree of aeration of any two parts of the disk. In all experiments of this type (entire disks with the halves insulated from each other) where large numbers of specimens were used two difficulties were met in making the measurements. Frequently the disk became folded backward at the point where the subumbrella ectoderm was removed, sometimes even bringing the exumbrella surfaces in contact. While this seemed in no way to interfere with regeneration, it frequently made accurate measurements impossible unless the specimen was first narcotized, as any attempt to unfold the active disk usually resulted in tearing the delicate regenerating tissue. This procedure involving the expenditure of so much time, all badly folded specimens were discarded. If the folding took place some days after the start of any series of experiments the specimen was discarded and the figures for the earlier stages were retained in the record.

The other most common source of difficulty in making the measurements arose on account of the tendency of the edge of the regenerating tissue to fuse with the edge of the old cut surface or with a more proximal part of the sheet of regenerating tissue. Whenever the edge of the thin sheet of new tissue became folded back sufficiently to touch any of the more proximal tissues fusion took place, so that a tube would be formed from the new tissue. When the folding involved only a small record of Table 1 - Cary '17

Table 1.—*Record of experiment 4a, started September 30, 1913 (for 1 to 5 days).*

[Forty disks each with all sense-organs removed from one side and with the halves insulated by removal of 2 strips of subumbrella ectoderm. The letter C after the measurement for any day indicates that the central cavity had been filled by the regenerated tissue.]

Specimen.		1 day.	2 days.	3 days.	4 days.	5 days.
No. 1	Half with S. O.	3.25	4.75	9.50 }C	.....	.....
	Half without S. O.	2.00	2.50	6.00	.....	.....
No. 2	Half with S. O.	2.50	4.00	5.00	9.00 }C	.....
	Half without S. O.	1.50	3.00	3.75	6.25	.....
No. 3	Half with S. O.	2.25	3.00	5.50	8.25 }C	.....
	Half without S. O.	1.75	2.00	3.25	5.00	.....
No. 4	Half with S. O.	2.75	4.00	5.75	14.75 }C	.....
	Half without S. O.	1.75	3.00	4.50	10.50	.....
No. 5	Half with S. O.	2.00	3.75	5.50	7.25 }C	9.00
	Half without S. O.	1.50	3.00	4.75	6.00	7.50
No. 6	Half with S. O.	1.50	3.00	3.75	5.00 }C	6.75 }C
	Half without S. O.	0.80	2.25	2.75	3.25	4.50
No. 7	Half with S. O.	3.75	5.00	5.75	7.50	.....
	Half without S. O.	1.75	2.00	3.00	5.00	.....
No. 8	Half with S. O.	3.50	4.00	4.00	9.50 }C	.....
	Half without S. O.	2.50	3.00	3.00	7.00	.....
No. 9	Half with S. O.	2.75	3.75	5.00	5.25	6.00 }C
	Half without S. O.	2.25	3.00	3.85	4.00	4.30
No. 10	Half with S. O.	3.25	5.50	8.25	9.50 }C	.....
	Half without S. O.	2.50	4.00	6.50	4.25	.....
No. 11	Half with S. O.	3.00	4.00	6.00	8.00 }C	.....
	Half without S. O.	2.15	3.00	4.00	6.25	.....
No. 12	Half with S. O.	2.50	5.00	6.50	7.50	9.00 }C
	Half without S. O.	1.75	3.75	4.50	5.25	6.50
No. 13	Half with S. O.	1.50	4.00	4.25	6.00	8.75 }C
	Half without S. O.	1.00	3.75	4.00	5.00	7.00
No. 14	Half with S. O.	4.00	5.00	6.80	7.00 }C	.....
	Half without S. O.	3.00	4.00	5.25	5.25	.....
No. 15	Half with S. O.	....	3.00	4.50	7.00	10.00
	Half without S. O.	....	2.00	3.50	5.50	7.00
No. 16	Half with S. O.	2.50	3.75	4.75	6.00	10.50 }C
	Half without S. O.	1.50	2.50	3.25	4.15	8.00
No. 17	Half with S. O.	2.75	3.75	6.25	7.75 }C	.....
	Half without S. O.	2.50	3.00	5.00	6.00	.....
No. 18	Half with S. O.	3.00	4.00	5.50	8.00 }C	.....
	Half without S. O.	2.00	3.00	3.75	5.75	.....
No. 19	Half with S. O.	2.75	3.50	4.25	6.00 }C	.....
	Half without S. O.	2.75	3.00	3.50	5.00	.....
No. 20	Half with S. O.	2.75	4.00	6.00	9.00	14.00 }C
	Half without S. O.	2.00	2.50	3.75	7.00	9.00
No. 21	Half with S. O.	2.50	3.50	4.50	7.00 }C	.....
	Half without S. O.	1.50	2.75	3.50	5.50	.....
No. 22	Half with S. O.	2.75	3.50	}Grown together.		
	Half without S. O.	1.50	2.50			
No. 23	Half with S. O.	3.00	4.25	5.75	9.00	.....
	Half without S. O.	2.00	3.00	4.25	8.25	.....
[illegible] 24	Half with S. O.	2.00	4.50	5.50 }C	.....	.....
	Half without S. O.	1.25	2.75	3.50	.....	.....
No. 25	Half with S. O.	2.25	4.75	8.00 }C	.....	.....
	Half without S. O.	1.50	3.15	5.50	.....	.....
No. 26	Half with S. O.	2.50	3.50	5.50	8.25 }C	.....
	Half without S. O.	1.50	2.25	3.75	6.50	.....
No. 27	Half with S. O.	2.00	4.50	6.75 }C	.....	.....
	Half without S. O.	1.50	3.75	5.00	.....	.....
No. 28	Half with S. O.	2.25	4.00	6.00 }C	.....	.....
	Half without S. O.	1.50	3.00	4.50	.....	.....

Table 1.—*Record of experiment 4a, started September 30, 1913 (for 1 to 5 days)*—Continued.

[Forty disks each with all sense-organs removed from one side and with the halves insulated by removal of 2 strips of subumbrella ectoderm. The letter C after the measurement for any day indicates that the central cavity had been filled by the regenerated tissue.]

Specimen.		1 day.	2 days.	3 days.	4 days.	5 days.
No. 29	Half with S. O.	2.50	3.75	4.50	Badly wrinkled.	
	Half without S. O.	2.00	3.00	3.50		
No. 30	Half with S. O.	3.00	3.55	3.75	4.75	7.00 C
	Half without S. O.	1.20	2.00	2.50	3.25	5.50
No. 31	Half with S. O.	2.00	3.00	5.00	7.00 C	.....
	Half without S. O.	1.00	2.25	3.50	5.00	.....
No. 32	Half with S. O.	2.00	3.00	6.50	10.75 C	.....
	Half without S. O.	1.25	2.00	4.00	7.50	.....
No. 33	Half with S. O.	1.75	2.50	4.50	6.00 C	.....
	Half without S. O.	0.50	1.00	2.50	4.00	.....
No. 34	Half with S. O.	2.00	4.00	6.00 C	.....	.....
	Half without S. O.	1.50	3.00	4.00	.....	.....
No. 35	Half with S. O.	3.00	Too badly wrinkled to measure new tissue.			
	Half without S. O.	2.00				
No. 36	Half with S. O.	3.75	8.25 C	....	.....	.....
	Half without S. O.	2.00	5.00	....	.....	.....
No. 37	Half with S. O.	2.75	4.00	7.00 C	.....	.....
	Half without S. O.	1.50	3.00	5.50	.....	.....
No. 38	Half with S. O.	1.50	2.25	Badly folded.		
	Half without S. O.	1.00	1.75			
No. 39	Half with S. O.	2.00	5.25	7.50 C	.....	.....
	Half without S. O.	1.25	3.00	5.25	.....	.....
No. 40	Half with S. O.	4.25	6.00 C	....	.....	.....
	Half without S. O.	3.00	4.75	....	.....	.....
Mean of all observations:						
-.46 ±7.64	Half with S. O.	2.65	4.05	5.75	7.63	9.00
1.73 ± 0.57	Half without S. O.	1.73	2.87	4.06	5.57	6.36

area separation could be easily accomplished, but if a considerable portion of the regenerating sheet was involved the specimen was rendered useless for further study.

The results of two typical experiments are shown in table 1. The measurements are in millimeters. The upper figure for each date shows the width of the sheet of tissue regenerated from the active half-disk, the lower figure the width of that regenerated from the inactive half-disk. When the sheet of new tissue had entirely closed over the cavity in the center of the disk the point of closure remained recognizable for at least a day, so that the measurements could readily be made for those disks that had become closed since the time of the last measurements. By the end of 24 hours after the new sheet of tissue was completed the point of closure would be shifted until it came to lie in the center of the disk.

The results from the 40 disks recorded in table 1 are shown in figure 6, in which the divisions along the abscissa show the time of regeneration in days, those along ordinate the amount of regeneration measured in millimeters. The record for each specimen is carried to the time of closure of the open circle in the center of the disk by the sheet of regenerated tissue.

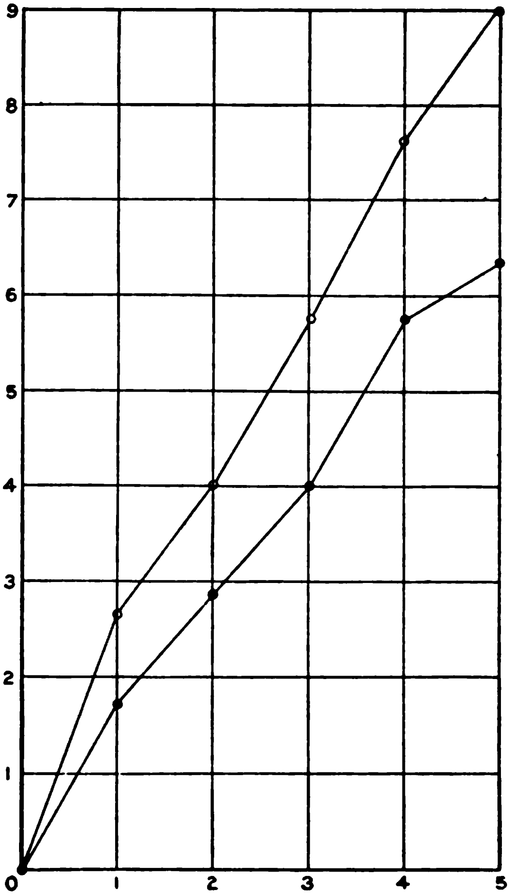


Fig. 6.—Showing relative rates of regeneration of the halves of 40 disks. The upper line represents the half-disks with sense-organs, the lower line represents those without sense-organs.

From the start of regeneration the new tissue produced from the side with its sense-organs intact is shown to be more rapid. In the early stage of regeneration this difference is more striking upon a cursory examination than in the later stages, although the actual difference on the rate of growth of new tissue changes only slightly during the entire period of regeneration. The proportion between the amounts of new tissue formed each day, taking the amount regenerated from the side without sense-organs as the unit, was respectively: First day 1:1.53; second day 1:1.44; third day 1:1.41; fourth day 1:1.38; fifth day 1:1.39.

The regeneration from the half without sense-organs is more regular, as is shown by the fact that for the mean of each day's observation the probable error is less for that half than for the one upon which the sense-organs remain. This result would be expected to follow from the fact that the inactive side was relieved from the influence of the marginal sense-organs, which would introduce many stimuli of varying intensity, all of which would have either a retarding or accelerating influence upon the processes of regeneration.

When the rates of regeneration of certain disks in table 1 are compared with one another, the cause of the uncertainty of the results obtained in experiments with entire disks is clearly shown. The closure of the open circle in disk 36, table 1, was complete in 2 days, while 5 days were necessary to complete the closure in specimen 30, table 1. Had the latter been the active disk and the former the inactive disk of a pair compared in an experiment with entire disks, the conclusion that an inactive disk sometimes regenerates more rapidly than an active one could not have been avoided.

EXPERIMENTS WITH ANESTHETICS.

In the previously described experiments it was shown that the active half of a medusa disk prepared as shown in figures 3 and 5 has a higher rate of regeneration than does the inactive half of the same specimen. While this point is clearly shown in all the experiments of the two types just mentioned, the results obtained by this method throw no light upon the nature of the control exercised by the marginal sense-organs as to whether it is exerted through the higher metabolism brought about by muscular activity or through some other less apparent metabolic process under the control of the sense-organs.

Two other types of experiments were undertaken to ascertain the nature of the nervous control. In the first set of experiments (type 3) disks prepared with insulated active and inactive halves (fig. 3) were allowed to regenerate in sea-water to which 15 per cent by volume of 0.6 m $MgSO_4$ had been added. In this solution the disks will live for an indefinite time and will for several hours retain the capacity to regain their normal activity within a few moments after being returned to fresh sea-water. Mayer (*op. cit.*) has shown that the effect of the magnesium in a weak $MgSO_4$ solution in sea-water is for a time confined almost entirely to the muscular tissues, while the nervous network is still capable of transmitting the impulse necessary for pulsation over an area submerged in the magnesium solution where no contraction of the muscles could be observed. When kept in the magnesium sea-water for a prolonged period the sense-organs become incapable of giving rise to the stimulus necessary for normal pulsation long before the nervous network loses its capacity for transmitting such a stimulus, so that a ring cut from a medusa disk and activated by a circuit wave of contraction will show by an indicator strip in sea-water (Mayer, *loc. cit.*, page 122) the transmission of the nervous impulse for some time after a ring retaining its sense-organs is no longer able to activate its indicator strip.

When medusa disks prepared with insulated active and inactive halves are put into the magnesium sea-water they lose their power of muscular movement within a few moments. Usually all of the disks float on the surface of the new solution for 20 to 30 minutes before they become adjusted to the abnormally dense medium; at the end of this period they settle to the bottom of the jar and remain completely relaxed throughout the experiment.

During the first 12 hours of an experiment, or as soon as the newly regenerated tissue became recognizable, regeneration is more rapid from the side on which the sense-organs are present. From that time on, the regeneration from the two halves is (within the limits of error of the measurements) about equal. The rate of regeneration of the half-disk with sense-organs fails to equal that of the half-disk without sense-organs. For both halves the rate was noticeably lower than that of the inactive half of a disk in normal sea-water. The lack of proper aeration commonly brought about through the pulsation of the active half-disk may account in part for the lower rate of regeneration, but there is unquestionably some more fundamental disturbance in the metabolic activity caused by the presence of the excess of Mg ions in the fluid.

The results from experiments of this type with the halves of 40 disks is given in table 2 and is shown graphically in figure 7.

TABLE 2.—*Record of experiments 12 and 12a (for 1 day to 9 days).*

[Forty disks each with insulated halves, one with sense-organs, the other without. Mg sea-water. The letter C after the measurement for any day indicates that the central cavity had been filled by the regenerated tissue.]

Specimen.		1 day.	2 days.	3 days.	4 days.	5 days.	6 days.	7 days.	8 days.	9 days.
No. 1	Half with S. O....	2.00	2.50	3.25	3.75	4.50	5.25	6.00	6.60	6.40
	Half without S. O.	0.80	1.40	2.00	2.75	3.40	4.20	4.80	5.70	6.55
No. 2	Half with S. O....	1.75	2.30	2.90	3.75	4.25	5.00	6.00	6.50	7.25
	Half without S. O.	0.90	1.50	1.75	2.50	3.25	4.00	4.75	5.25	6.00
No. 3	Half with S. O....	1.00	1.50	2.75	3.75	4.50	5.00	5.75	6.50	7.00
	Half without S. O.	0.50	0.75	1.25	2.00	3.00	3.75	4.50	5.25	6.25
No. 4	Half with S. O....	1.50	2.25	3.00	3.50	4.25	5.00	5.75	6.50	7.25
	Half without S. O.	1.00	1.50	2.00	2.75	3.25	3.75	4.50	5.25	6.00
No. 5	Half with S. O....	1.75	2.25	3.00	3.75	4.50	5.25	5.75	6.25	7.00
	Half without S. O.	1.00	1.50	2.25	2.00	3.50	4.00	4.50	5.00	5.75
No. 6	Half with S. O....	2.00	2.75	3.50	4.25	5.25	5.75	6.25	7.00	C
	Half without S. O.	1.25	1.75	2.50	3.00	3.75	4.25	4.75	5.75	
No. 7	Half with S. O....	1.00	2.00	3.00	3.75	4.75	5.50	6.25	7.00	7.50
	Half without S. O.	0.75	1.25	2.00	2.50	3.25	4.00	4.50	5.25	5.75
No. 8	Half with S. O....	1.25	1.50	2.25	3.00	4.00	4.75	5.50	6.25	7.00
	Half without S. O.	0.75	1.00	1.25	1.75	3.50	3.25	3.75	4.50	5.25
No. 9	Half with S. O....	2.25	2.75	3.50	4.00	4.75	5.50	6.25	7.25	C
	Half without S. O.	1.00	1.50	2.25	2.75	3.25	4.00	4.50	5.25	
No. 10	Half with S. O....	1.50	2.25	3.00	3.50	4.25	5.00	5.50	6.25	7.25
	Half without S. O.	0.50	1.00	1.75	2.25	3.00	3.75	4.50	5.25	6.00
No. 11	Half with S. O....	1.00	1.75	2.50	3.50	4.25	5.00	5.75	6.50	7.25
	Half without S. O.	0.50	1.00	1.75	2.50	3.25	4.00	4.50	5.25	6.00
No. 12	Half with S. O....	2.50	3.25	4.00	4.75	5.50	6.25	7.00	7.75	C
	Half without S. O.	1.25	1.75	2.25	2.75	3.25	4.00	4.50	5.25	
No. 13	Half with S. O....	1.75	2.25	3.00	3.75	4.50	5.25	5.75	6.50	7.25
	Half without S. O.	0.50	1.25	1.75	2.50	3.25	4.00	4.75	5.50	6.00
No. 14	Half with S. O....	0.75	1.50	2.25	2.75	3.75	4.50	5.25	6.25	7.25
	Half without S. O.	0.25	0.75	1.50	2.25	3.00	3.75	4.50	5.25	6.25
No. 15	Half with S. O....	1.50	2.00	2.75	3.75	4.50	5.25	6.00	6.75	7.25
	Half without S. O.	0.50	1.25	1.75	2.50	3.25	4.00	4.75	5.25	6.00
No. 16	Half with S. O....	2.00	2.75	3.25	4.00	4.50	5.25	6.00	6.50	7.25
	Half without S. O.	0.75	1.25	1.75	2.50	3.25	4.00	4.75	5.25	6.00

TABLE 2.—*Record of experiments 12 and 12a (for 1 day to 9 days)*—Continued.

[Forty disks each with insulated halves, one with sense-organs, the other without. Mg. sea-water. The letter C after the measurement for any day indicates that the central cavity had been filled by the regenerated tissue.]

Specimen.		1 day.	2 days.	3 days.	4 days.	5 days.	6 days.	7 days.	8 days.	9 days.
No. 17	Half with S. O....	1.75	2.50	3.25	4.00	4.75	5.25	6.00	6.75	7.50
	Half without S. O.	0.50	1.25	2.00	2.50	3.25	4.00	4.75	5.25	6.00
No. 18	Half with S. O....	2.25	3.00	3.75	4.50	5.00	6.00	7.00	C	....
	Half without S. O.	1.00	1.50	2.25	2.75	3.50	4.25	5.25		....
No. 19	Half with S. O....	2.75	3.25	4.50	5.25	6.00	6.75	7.50	C	....
	Half without S. O.	1.50	2.00	2.50	3.25	3.75	4.25	5.00		....
No. 20	Half with S. O....	1.50	2.25	3.00	3.75	4.50	5.50	6.25	7.00	7.75
	Half without S. O.	0.75	1.50	2.00	2.50	3.25	4.00	4.75	5.25	6.00
No. 21	Half with S. O....	3.00	3.50	4.25	4.75	5.25	6.00	6.75	7.50	C
	Half without S. O.	1.50	2.00	2.50	3.25	3.75	4.50	5.25	6.00	
No. 22	Half with S. O....	2.25	2.75	3.25	3.75	4.50	5.25	6.00	6.50	7.25
	Half without S. O.	1.00	1.50	2.00	2.50	3.25	4.00	4.75	5.50	6.25
No. 23	Half with S. O....	1.50	2.25	3.00	3.75	4.25	5.00	5.75	6.50	7.25
	Half without S. O.	0.75	1.25	2.00	2.75	3.50	4.25	5.00	5.75	6.00
No. 24	Half with S. O....	2.00	2.50	3.25	3.75	4.50	5.00	5.75	6.25	7.00
	Half without S. O.	0.75	1.50	2.25	1.75	3.25	3.75	4.50	5.00	5.75
No. 25	Half with S. O....	1.25	2.00	2.75	3.50	4.75	5.25	6.00	6.75	7.50
	Half without S. O.	0.50	1.00	1.50	2.25	2.00	3.50	4.25	5.00	6.00
No. 26	Half with S. O....	1.75	2.25	3.00	4.00	4.75	5.25	6.00	7.00	8.00
	Half without S. O.	0.50	1.00	1.50	2.50	3.25	3.75	4.50	5.25	6.25
No. 27	Half with S. O....	2.50	3.25	4.00	4.75	5.50	6.50	7.50	8.25	C
	Half without S. O.	1.00	1.75	2.50	3.25	4.00	4.75	5.50	6.25	
No. 28	Half with S. O....	2.25	3.00	3.50	4.00	4.75	5.50	6.25	7.00	7.75
	Half without S. O.	1.00	1.75	2.25	3.00	3.50	4.00	4.75	5.25	6.00
No. 29	Half with S. O....	1.75	2.50	2.00	3.75	4.25	5.00	5.75	6.50	7.25
	Half without S. O.	1.00	1.75	2.25	2.75	3.50	4.25	5.00	5.75	6.50
No. 30	Half with S. O....	2.75	3.25	4.00	5.00	6.00	6.75	7.50	C	....
	Half without S. O.	1.75	1.50	2.25	3.00	3.75	4.00	5.00		....
No. 31	Half with S. O....	2.25	3.00	3.75	4.50	5.25	6.00	6.75	7.50	C
	Half without S. O.	1.00	1.50	2.25	3.00	3.75	4.50	5.25	6.00	
No. 32	Half with S. O....	1.50	2.50	3.25	3.75	4.50	5.25	6.00	6.75	7.50
	Half without S. O.	0.50	1.25	2.00	2.75	3.50	4.25	5.00	5.75	6.25
No. 33	Half with S. O....	2.00	2.75	3.25	4.00	4.50	5.25	6.00	6.75	7.50
	Half without S. O	0.75	1.50	2.00	2.75	3.25	4.00	4.75	5.50	6.25
No. 34	Half with S. O....	1.00	1.75	2.50	3.25	4.00	5.00	5.75	6.50	7.25
	Half without S. O.	0.25	1.00	1.75	2.00	2.75	3.50	4.25	5.00	5.75
No. 35	Half with S. O....	1.75	2.50	3.25	3.75	4.50	5.26	6.00	6.75	7.50
	Half without S. O.	1.00	1.50	2.25	2.75	3.50	4.25	4.75	5.50	6.25
No. 36	Half with S. O....	2.25	3.00	3.50	4.00	4.50	5.25	6.00	6.50	7.25
	Half without S. O.	1.00	1.75	2.50	3.25	4.00	4.75	5.50	6.00	6.75
No. 37	Half with S. O....	1.50	2.25	3.00	3.75	4.50	5.25	6.00	6.75	7.75
	Half without S. O.	0.75	1.50	2.25	2.75	3.50	4.25	5.00	5.75	6.50
No. 38	Half with S. O....	2.75	3.50	4.25	5.00	5.75	6.50	7.25	8.00	C
	Half without S. O.	1.00	1.75	2.50	3.25	4.00	4.75	5.50	6.00	
No. 39	Half with S. O....	2.25	3.00	3.50	4.25	5.00	5.50	6.00	6.75	7.50
	Half without S. O.	0.75	1.50	2.25	3.00	3.75	4.50	5.00	5.75	6.50
No. 40	Half with S. O....	1.50	2.25	3.00	3.50	4.25	5.00	5.75	6.50	7.25
	Half without S. O.	0.50	1.25	2.75	2.75	3.50	4.25	5.00	5.75	6.50
Mean of all observations:										
	Half with S. O....	2.00	2.35	2.92	3.55	4.57	5.17	5.94	6.47	7.40
	Half without S. O.	0.85	1.43	2.00	2.85	3.45	4.23	4.87	5.74	6.47

Experiments with KCN or chloroform dissolved in sea-water did not give satisfactory results. In both these solutions the tissues of the medusa underwent rapid disintegration if the amount of the reagent present was sufficient to bring about any noticeable effect upon the activity of the sense-organs.

When medusæ are treated with a weak solution of oxalic acid in magnesium-free artificial sea-water it is possible to destroy the activity of the sense-organs for a considerable time without seriously injuring the other tissues. In all my experiments, however, recovery of functional activity by the sense-organs took place within 24 hours if the oxalic-acid solution was of such a strength that the ectodermal tissues were not injured. In such experiments there was at first an equal rate of regeneration for each of the halves of a disk until the sense-organs regained their functional activity, after which the half-disk with sense-organs regenerated most rapidly.

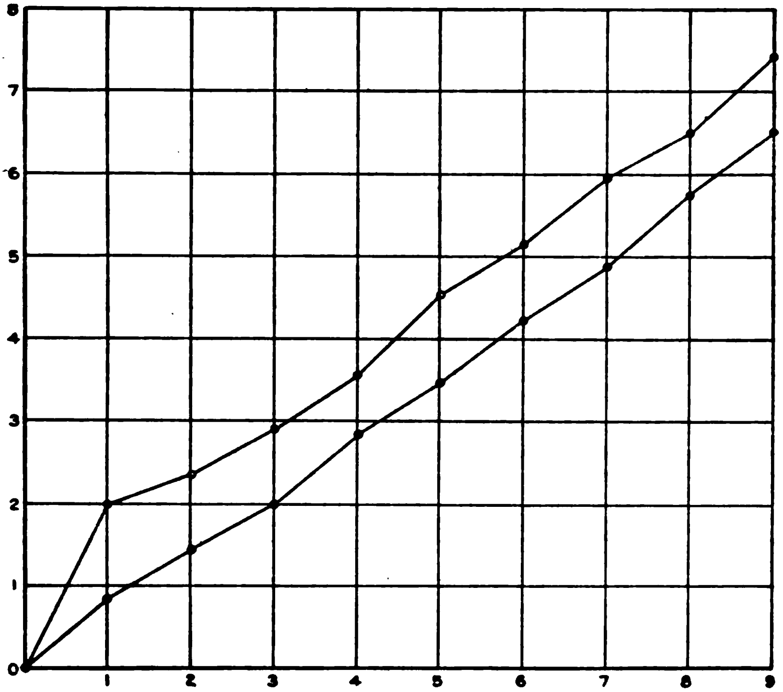


Fig. 7.—Showing the rates of regeneration of active and inactive half-disks regenerating in sea-water to which has been added 15 parts of 0.6 m $MgSO_4$. The upper line represents the half with sense-organs; the lower line represents those without sense-organs. The divisions along the ordinate represent the amount of regeneration in millimeters; those along the abscissa the time of regeneration in days.

The experiments with Mg solutions show that there is an influence of the sense-organs on the rate of regeneration which is apparently exercised for a considerable time after muscular activity has been suppressed. It was impossible, however, by this method to differentiate with any certainty between the two effects, since there is no visual means of ascertaining the exact time at which the sense-organs lose their power of sending out the stimuli necessary for normal contraction. Since, as was shown by Mayer (*op. cit.*), it is possible to maintain a circuit wave of contraction in the muscles of a half-disk without sense-organs by making a series of cuts by which an endless labyrinth of subumbrella tissue is formed (figs. 4 and 5), the part played by muscular activity and that by the stimuli from the sense-organs can be directly compared. In such experiments all the sense-organs were removed from the medusa disks, the halves insulated, and a circuit wave of contraction started by an induction shock in a labyrinth cut in the muscle-tissues of one of its halves. Once established, the contraction wave would be maintained throughout the course of an experiment unless interrupted by an unusually strong stimulus through some accident in handling. When interrupted in this way the circuit wave could be established again by renewed electrical stimulation. The amplitude of the contraction wave becomes gradually reduced as time goes on, but there is little variation in its rate. When the rates of regeneration of the halves of any disk prepared in this manner are compared, it is found that the half in which the circuit wave is maintained regenerates slightly faster than the inactive one. This difference in rate is, however, very much less than between the halves of a disk from one-half of which the sense-organs have been removed (compare figs. 6 and 8), although the activated disk pulsates from 3 to 10 times as fast as the one under the control of the sense-organs.

TABLE 3.—*Record for 1 to 9 days.*

[Forty specimens from which all of the sense-organs had been removed and the subumbrella muscles of one half activated by a circuit wave of contraction (fig. 8). The letter C after the measurement for any day indicates that the central cavity had been filled by the regenerated tissue.]

Specimen.		1 day.	2 days.	3 days.	4 days.	5 days.	6 days.
No. 1	Activated half..........	2.00	3.25	4.50	6.25	7.25	8.00
	Half without S. O.......	1.75	2.75	4.00	5.50	6.50	7.25
No. 2	Activated half..........	2.50	3.25	4.75	6.50	8.00	C
	Half without S. O.......	2.00	3.00	4.25	6.00	7.50	
No. 3	Activated half..........	1.75	3.00	4.50	6.00	7.00	8.25
	Half without S. O.......	1.50	2.25	3.25	5.25	6.25	7.00
No. 4	Activated half..........	1.50	2.75	4.00	6.00	7.25	8.25
	Half without S. O.......	1.25	2.50	3.50	5.25	6.00	7.00
No. 5	Activated half..........	2.25	3.25	4.50	6.25	7.00	8.25
	Half without S. O.......	1.75	2.75	4.00	5.50	6.25	7.25
No. 6	Activated half..........	2.00	3.25	4.75	6.00	7.50	8.50
	Half without S. O.......	1.75	2.75	4.00	5.50	6.50	7.25
No. 7	Activated half..........	2.75	4.00	5.00	6.50	8.00	C
	Half without S. O.......	2.25	3.25	4.25	5.50	7.00	
No. 8	Activated half..........	3.00	4.00	5.25	6.50	8.00	C
	Half without S. O.......	2.50	3.00	4.50	5.75	7.25	
No. 9	Activated half..........	1.00	2.25	3.50	5.25	6.50	8.00
	Half without S. O.......	0.75	1.75	3.00	3.75	5.75	7.25
No. 10	Activated half..........	1.75	3.00	4.25	6.00	7.50	8.25
	Half without S. O.......	1.50	2.50	3.50	5.00	6.25	7.00
No. 11	Activated half..........	2.50	3.25	4.50	6.25	7.25	8.00
	Half without S. O.......	2.25	2.75	4.00	3.75	6.75	7.50
No. 12	Activated half..........	3.50	4.25	5.25	6.75	8.25	C
	Half without S. O.......	3.25	3.75	4.75	6.00	7.50	

TABLE 3.—*Record for 1 to 9 days*—Continued.

Forty specimens from which all of the sense-organs had been removed, and the subumbrella muscles of one half activated by a circuit wave of contraction (fig. 8). The letter C after the measurement for any day indicates that the central cavity had been filled by the regenerated tissue.]

Specimen.		1 day.	2 days.	3 days.	4 days.	5 days.	6 days.
No. 13	Activated half..........	1.25	2.50	4.25	6.00	7.50	8.50
	Half without S. O.......	1.00	2.25	4.00	5.50	6.75	7.25
No. 14	Activated half..........	2.00	3.25	4.50	6.25	7.25	8.25
	Half without S. O.......	1.75	2.75	4.00	5.75	6.50	7.25
No. 15	Activated half..........	2.25	3.50	4.50	6.50	8.00	}C
	Half without S. O.......	2.00	3.00	4.00	6.00	7.25	
No. 16	Activated half..........	1.00	2.50	4.25	6.00	7.25	8.25
	Half without S. O.......	0.75	2.25	4.00	5.00	6.25	7.00
No. 17	Activated half..........	1.75	3.00	4.25	6.00	7.25	8.00
	Half without S. O.......	1.50	2.50	3.75	5.50	6.75	7.50
No. 18	Activated half..........	2.50	3.50	4.50	6.50	8.50	}C
	Half without S. O.......	2.25	3.00	4.00	6.00	7.75	
No. 19	Activated half..........	1.25	2.75	4.00	6.00	7.00	7.75
	Half without S. O.......	1.00	2.50	3.75	5.75	6.75	7.50
No. 20	Activated half..........	2.00	3.25	4.50	6.00	7.25	8.00
	Half without S. O.......	1.75	2.75	4.00	5.75	6.50	7.25
No. 21	Activated half..........	1.75	3.00	4.50	6.25	7.25	8.00
	Half without S. O.......	1.50	2.75	4.00	5.50	6.50	7.25
No. 22	Activated half..........	2.50	3.25	4.50	6.00	7.00	8.25
	Half without S. O.......	2.00	2.75	4.00	5.50	6.25	7.25
No. 23	Activated half..........	2.00	3.00	4.50	6.00	7.00	8.00
	Half without S. O.......	1.75	2.75	4.00	5.25	6.25	7.25
No. 24	Activated half..........	3.00	3.75	5.00	6.25	8.00	}C
	Half without S. O.......	2.50	3.25	4.25	5.50	7.25	
No. 25	Activated half..........	1.50	2.50	4.25	5.75	7.00	8.00
	Half without S. O.......	1.25	2.25	4.00	5.50	6.50	7.50
No. 26	Activated half..........	1.00	2.50	4.50	6.25	7.00	8.00
	Half without S. O.......	0.75	2.25	4.00	5.50	6.25	7.25
No. 27	Activated half..........	2.00	3.25	4.50	6.50	7.25	8.00
	Half without S. O.......	1.75	3.00	3.75	5.75	6.50	7.25
No. 28	Activated half..........	2.25	3.25	4.50	6.25	7.00	8.00
	Half without S. O.......	1.75	2.75	4.00	5.50	6.25	7.25
No. 29	Activated half..........	2.00	3.00	4.75	6.25	7.25	8.00
	Half without S. O.......	1.75	2.75	4.00	5.50	6.25	7.00
No. 30	Activated half..........	1.50	2.50	4.00	6.00	7.00	8.25
	Half without S. O.......	1.25	2.25	3.75	5.50	6.50	7.25
No. 31	Activated half..........	2.00	3.00	4.50	6.00	7.25	8.00
	Half without S. O.......	1.50	2.50	4.00	5.25	6.25	7.00
No. 32	Activated half..........	2.75	3.50	4.75	6.75	7.50	8.50
	Half without S. O.......	2.25	3.00	4.00	5.75	6.75	7.25
No. 33	Activated half..........	4.00	5.75	7.75	8.75	}C	....
	Half without S. O.......	3.50	4.75	7.00	7.75		....
No. 34	Activated half..........	2.00	3.50	5.00	6.50	8.00	}C
	Half without S. O.......	1.75	3.00	4.25	5.75	7.50	
No. 35	Activated half..........	1.25	2.50	4.00	6.00	7.50	8.50
	Half without S. O.......	1.00	2.25	3.75	5.75	6.50	7.25
No. 36	Activated half..........	1.00	2.50	4.25	6.00	7.25	8.00
	Half without S. O.......	0.75	2.00	3.75	5.50	6.75	7.50
No. 37	Activated half..........	1.50	3.00	4.75	6.25	7.25	8.00
	Half without S. O.......	1.25	2.75	4.25	5.50	6.50	7.25
No. 38	Activated half..........	2.00	3.00	4.25	6.00	7.25	8.25
	Half without S. O.......	1.75	2.75	4.00	5.50	6.50	7.25
No. 39	Activated half..........	1.00	2.25	4.00	6.00	7.00	8.25
	Half without S. O.......	0.75	2.00	3.75	5.25	6.25	7.50
No. 40	Activated half..........	2.00	3.00	4.50	6.50	7.50	8.50
	Half without S. O.......	1.75	2.50	4.00	5.50	6.25	7.00
Mean of all observations:							
	Activated half..........	2.00	3.27	4.67	6.15	7.23	8.12
	Half without S. O.......	1.73	2.87	4.06	5.57	6.36	7.27

The amount of activity and metabolism in the muscles if they have any noticeable influence on the rate of regeneration ought to produce a clearly demonstrable result, but as shown by the data in table 3 and figure 8 the difference is relatively small. From the point of view of the chemical nature of metabolism (including regeneration) the difference in temperature might conceivably be sufficient to account for the observed difference in rate of regeneration. The half-disks in which the circuit wave is maintained show a greater regularity in the rate of regeneration than do the active disks recorded in table 1.

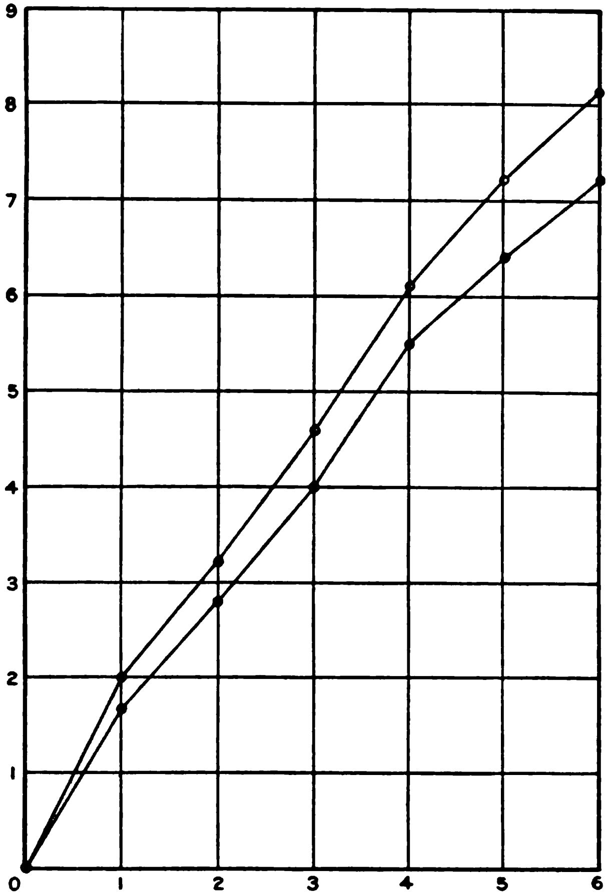


Fig. 8.—Showing rates of regeneration of activated and inactive half-disks. The upper line represents the activated and the lower line the inactive specimens. The divisions along the abscissa represent regeneration in millimeters; those along the ordinate represent time in days.

Further demonstration of the influence of the sense-organs on the rate of regeneration is furnished in another series of experiments in which the two insulated halves of a disk are compared, one of which is contracting normally under the influence of its sense-organs, while all the sense-organs are removed from the other half and a circuit wave of contraction maintained in a labyrinth of its subumbrella muscles. In a disk prepared in this manner the rate of pulsation of the activated half will vary from 3 to 10 times that of the normally contracting half.

The comparative rates of regeneration for 40 disks under these conditions are shown in table 4 and figure 9.

TABLE 4.—*Record for 1 to 5 days.*

[Forty specimens, one half of each one pulsating under the control of its sense-organs, while a circuit wave of contraction was maintained in the subumbrella muscles of the other half (fig. 10). The letter C after the measurement for any day indicates that the central cavity had been filled by the regenerated tissue.]

Specimen.		1 day.	2 days.	3 days.	4 days.	5 days.
No. 1	Half with S. O......	3.00	4.25	5.75	7.50	9.00
	Activated half......	2.25	3.25	4.50	6.00	7.50
No. 2	Half with S. O......	1.75	3.50	5.50	7.25	9.00
	Activated half......	1.50	3.25	5.25	7.00	8.00
No. 3	Half with S. O......	4.00	5.50	6.75	8.25	C
	Activated half......	3.50	4.75	5.50	7.50	
No. 4	Half with S. O......	2.50	4.25	5.50	7.50	9.50
	Activated half......	2.00	3.75	4.75	6.75	8.00
No. 5	Half with S. O......	2.00	4.00	5.50	7.50	9.00
	Activated half......	1.50	3.25	4.50	6.00	7.50
No. 6	Half with S. O......	2.75	4.25	5.75	7.75	9.25
	Activated half......	2.00	3.50	4.75	6.50	7.50
No. 7	Half with S. O......	2.50	4.00	5.50	7.25	8.75
	Activated half......	2.00	3.25	4.75	6.25	7.75
No. 8	Half with S. O......	2.25	4.25	5.75	7.50	9.00
	Activated half......	1.75	3.00	4.25	6.00	7.50
No. 9	Half with S. O......	3.00	4.50	5.75	7.75	9.25
	Activated half......	2.50	3.75	5.00	6.50	7.50
No. 10	Half with S. O......	1.50	3.00	5.00	7.00	9.00
	Activated half......	1.25	2.75	4.50	6.25	8.00
No. 11	Half with S. O......	2.25	4.00	5.50	7.00	9.00
	Activated half......	1.75	3.25	4.75	6.25	7.50
No. 12	Half with S. O......	3.00	4.75	5.50	7.75	9.25
	Activated half......	2.25	3.25	4.50	6.00	7.25
No. 13	Half with S. O......	2.50	4.00	5.75	7.25	9.00
	Activated half......	2.00	3.50	4.75	6.25	7.25
No. 14	Half with S. O......	2.75	4.25	5.75	7.75	9.25
	Activated half......	2.25	3.50	4.50	6.50	7.75
No. 15	Half with S. O......	3.00	4.25	5.75	8.00	9.25
	Activated half......	2.25	3.25	4.75	6.25	7.50
No. 16	Half with S. O......	4.00	6.00	7.75	9.50	C
	Activated half......	3.25	5.25	7.00	8.25	
No. 17	Half with S. O......	2.50	4.25	5.50	7.25	9.00
	Activated half......	2.00	3.25	4.75	6.50	7.75
No. 18	Half with S. O......	2.00	4.00	5.75	7.25	9.25
	Activated half......	1.50	3.50	4.75	6.00	7.50
No. 19	Half with S. O......	2.75	4.25	5.50	7.25	8.75
	Activated half......	2.00	3.25	4.50	6.50	8.00
No. 20	Half with S. O......	2.25	4.00	5.75	7.50	9.00
	Activated half......	1.50	3.25	4.75	6.25	7.25
No. 21	Half with S. O......	3.00	4.50	6.00	9.00	C
	Activated half......	2.50	4.00	4.75	7.50	
No. 22	Half with S. O......	2.25	4.00	6.00	8.00	C
	Activated half......	2.00	3.75	5.50	7.75	

TABLE 4.—*Record for 1 to 5 days*—Continued.

[Forty specimens, one half of each one pulsating under the control of its sense-organs, while a circuit wave of contraction was maintained in the subumbrella muscles of the other half (fig. 10). The letter C after the measurement for any day indicates that the central cavity had been filled by the regenerated tissue.]

Specimen.		1 day.	2 days.	3 days.	4 days.	5 days.
No. 23	Half with S. O......	2.75	4.25	6.00	7.50	9.00
	Activated half......	2.25	3.50	5.00	6.25	7.50
No. 24	Half with S. O......	2.50	4.00	5.75	7.75	9.25
	Activated half......	2.00	3.25	4.75	6.25	7.50
No. 25	Half with S. O......	1.25	3.50	5.50	7.25	9.00
	Activated half......	1.00	3.00	4.75	6.00	7.00
No. 26	Half with S. O......	3.00	5.00	6.50	9.00	C
	Activated half......	2.25	3.50	5.00	7.50	
No. 27	Half with S. O......	4.00	6.00	9.00	C	....
	Activated half......	3.25	5.50	8.25		....
No. 28	Half with S. O......	2.75	4.00	5.75	8.00	9.50
	Activated half......	2.00	3.25	4.75	6.50	8.00
No. 29	Half with S. O......	1.75	3.75	5.50	7.25	8.50
	Activated half......	1.00	3.25	4.25	6.00	7.00
No. 30	Half with S. O......	2.50	4.00	5.75	7.75	9.00
	Activated half......	2.00	3.00	4.75	6.50	7.25
No. 31	Half with S. O......	1.50	3.50	5.50	7.50	8.75
	Activated half......	1.00	3.00	4.75	6.25	7.25
No. 32	Half with S. O......	3.00	4.25	5.75	7.50	8.50
	Activated half......	2.25	3.50	4.75	6.25	7.50
No. 33	Half with S. O......	2.25	3.75	5.50	7.50	9.00
	Activated half......	1.75	3.00	4.25	5.75	7.25
No. 34	Half with S. O......	2.00	3.50	5.50	7.75	9.00
	Activated half......	1.75	2.75	4.75	6.25	7.00
No. 35	Half with S. O......	2.75	4.00	5.50	7.75	9.25
	Activated half......	2.00	3.25	4.75	6.25	7.50
No. 36	Half with S. O......	3.00	4.00	5.75	8.00	9.25
	Activated half......	2.25	3.25	4.75	6.50	7.00
No. 37	Half with S. O......	3.50	5.00	7.25	9.25	C
	Activated half......	2.25	3.50	5.00	7.50	
No. 38	Half with S. O......	2.75	4.00	5.75	7.75	9.25
	Activated half......	2.00	3.25	4.50	6.25	7.00
No. 39	Half with S. O......	2.00	3.75	5.50	7.75	8.75
	Activated half......	1.50	3.25	4.75	6.25	7.25
No. 40	Half with S. O......	4.00	6.00	8.75	C	....
	Activated half......	2.75	4.50	7.50		....
Mean for all observations:						
	Half with S. O......	2.65	4.05	5.75	7.63	9.00
	Activated half......	2.00	3.27	4.67	6.15	7.23

As was pointed out previously, the stimulus for pulsation originates, at any given time, from a single sense-organ, and pulsation will be continued at nearly its normal rate by a medusa upon which a single sense-organ remains. To test the efficacy of a single sense-organ to control activities other than muscular contraction a series of disks prepared as above described (except that each "active" half-disk had a single sense-organ) were used in a parallel series of regeneration experiments. In all instances such active disks showed an unusual irregularity in the rate of pulsation, since there was present no other nerve-center to take up the initiation of impulses causing pulsation whenever the rate of discharge of the single organ fell below normal.

There was noticeable an indefinite sort of rhythm in the rate of pulsation, as if the sense-organ underwent periods of fatigue from which it was enabled to recover after having given off the stimuli for pulsation for a time at a rate considerably lower than that observed previous to the removal of the other centers. Those periods of depression in the pulsation-rate were of such short duration that they could not be distinguished in the amount of tissue regenerated, if, as would seem probable, the sense-organs were affecting other metabolic activities in a similar manner.

In all experiments of this type the results were of the same nature as in those when the sense-organs on the activated half-disks had not been disturbed. The amount of time necessary to bring about the closing of the cavity in which regeneration was measured was not sufficiently different from that in specimens where the active half-disk retained its sense-organs to indicate any serious disturbance in metabolic activity. The different specimens in these, as in the previous series, showed great variations in physiological activity (see tables 1, 2, 3, and 4), but there was nothing strikingly characteristic about the behavior of the specimens with a single sense-organ.

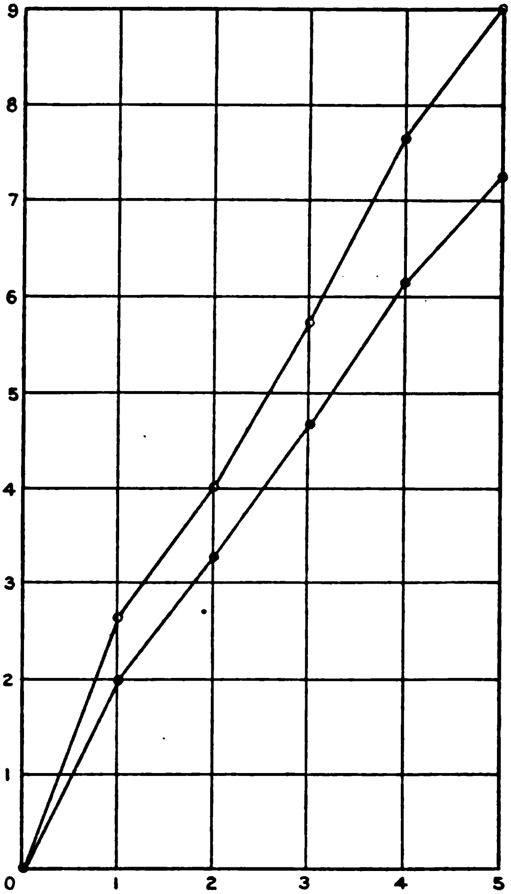


Fig. 9.—Showing rates of regeneration of active and activated half-disks. The divisions along the ordinate represent regeneration in millimeters; those along the abscissa represent time in days.

EFFECT OF TIME INTERVAL BETWEEN SEPARATION OF DISKS INTO HALVES AND REMOVAL OF SENSE-ORGANS.

In all the experiments on regeneration involving half-disks which retained their sense-organs it was noticeable that the difference in rate of regeneration from the two halves was most marked during the first day (see figures 6, 7, 8, 9). After that time the rates of the two halves gradually became more nearly equal, until at the end of an experiment, if the amount to be regenerated was large, there was only a slight difference between the rates of the two halves.

In a series of experiments designed to test the importance of the time factor in the difference in the rate of regeneration, sets of 10 disks each were separated into halves and the sense-organs were removed from one of the halves of each pair at intervals of 2 hours. It was soon found that no effect was noticeable if the centers were removed in less than 18 hours, and in most experiments 25 or more hours were necessary in order that the rate of regeneration should be the same from each half of a disk. When the second operation was made in less than 24 hours from the time of dividing the disk it was impossible to distinguish between such specimens and the controls from which half of the sense-organs were removed at the time of the first operation.

Table 5 shows the effect of the removal of the sense-organs from a half-disk of a pair at different intervals after the separation of the disks into halves, each series consisting of 10 specimens.

TABLE 5.—*Effect upon regeneration of the time interval between the separation of a disk into halves and the removal of the sense-organs from one half.*

Series.	First operation.	Rhopalia removed.	Results.	
No. 1...	9^{h} 45^{m} a. m. July 22	7 a. m. July 23	Half with sense-organ fastest.	21¼
No. 2...	8^{h} 00^{m} a. m. July 24	7 p. m. July 24	Half with sense-organ fastest.	11
No. 3...	7^{h} 15^{m} a. m. July 25	8 a. m. July 26	Regeneration equal.	24¾
No. 4...	8^{h} 00^{m} a. m. July 25	4 p. m. July 25	Half with sense-organ fastest.	8
No. 5...	7^{h} 00^{m} a. m. July 26	8 a. m. July 27	Regeneration equal.	25

As shown above, the line of demarcation between the time when no influence of the sense-organs will be appreciable and that after which their removal has no effect is sharply drawn at an interval of about 25 hours. In each series of disks there was observed the same irregularity in physiological activity that was shown among the members of any large series. The individuals of the highest physiological activity, as shown in the rate of regeneration, were the first to show the effects of the sense-organs. In any series those specimens from which the sense-organs could first be removed with the assurance that their influence would be shown on the later stages of regeneration could be distinguished after 12 hours by observing the rate at which regeneration was taking place.

The foregoing data seem to show conclusively that there is a clearly marked influence of the sense-organs upon the rate of regeneration in *Cassiopea*. There is no evidence, however, that the presence of this influence is necessary for the formation of normal structures in regeneration, as Herbst (1896 and 1899) concluded from his experiments on decapods; or as maintained by several of the earlier workers upon regeneration in platodes, and by Walter (1911) for *Triton*. The second of these special cases has been shown by the studies of Morgan, Child, and Goldfarb to be conditioned by influences other than the presence of the nerve-centers. The work of Steele has shown also that in several species of crustacea the removal of an eye-stalk is not followed by the regeneration of a heteromorphic structure.

While none of these workers has laid any stress on the fact that the nervous system exerts an influence on the rapidity of the early stages of regeneration, it has been noted in several instances that the initial stages of regeneration are more rapid in the control animals than in those from which the nervous system has been removed. Thus, Goldfarb (*op. cit.*, page 664) states that in salamanders the hind limb develops more slowly on the side from which the dorsal ganglia innervating the leg had been removed than does that of the opposite side in which the ganglia remained when the spinal cord had already been removed. In a table on pages 665 and 666 he shows that this result was observed in all the specimens recorded save two, in one of which the regeneration was, at the time of measurement, equal for both legs, while in a single specimen the regeneration was most rapid from the side from which the ganglia were removed.

In tadpoles from which the caudal portion of the spinal cord had been removed regeneration of the tail took place more slowly than in the control animals in which the cord was uninjured (p. 672). Again, concerning earthworms from which the head had been cut off and several millimeters of the ventral cord removed he says (p. 708): "The head regenerates rather later in these operated animals than in control animals." In the regeneration of the arms of the starfish (p. 711) a similar observation is recorded.

Goldfarb, however, is of the opinion that "any severe injury * * *, whether involving the nerves or any other tissue, retards regeneration." Stockard, on the other hand, concluded that in *Cassiopea*, as Morgan had already shown for a considerable number of animals, the rate of regeneration increased in proportion to the extent of injury and that the deeper the cut—*i.e.*, the nearer to the center of the disk—the faster would be the following regeneration.

In my experiments the amount of tissue removed from the margin of each half of any disk was the same. The differences in result observed were, therefore, due to the difference in kind, not in quantity of tissue removed. As recorded previously, the difference in rate of regeneration is in *Cassiopea* greatest in the early stages and gradually declines throughout its course, at least through the periods followed in these experiments. Goldfarb's observations appear also to show a similar course of events in earthworms, starfish, and amphibians. This result, on the other hand, is opposed to the conclusion of Child (1910) that "as most experiments not only on the Turbellaria, but on other forms, indicate, it is probable that the early stages of the formation of new tissue are largely or wholly independent of the nervous system." A later part of the same statement is in perfect accord with my result, as follows: "But it is difficult to understand how the nervous system of an adult animal could fail to affect the amount and rapidity of growth in a regenerating part composed largely of muscles and sense-organs. Absence of such an effect would be in direct opposition to the well-established fact of the functional influence of the nervous system on various parts of the organism."

Morgulis (1912) concludes from his experiments on brittle-stars that the presence at the cut surface of the radial nerve, either with or without its being in continuity with the remainder of the nervous system, is a "conditio sine qua non" for normal regeneration and that the presence—purely as a mechanical matter, not the functional activity—of the nerve is the important factor in regeneration. This is in direct opposition to my results. A study of the figures illustrating his paper shows, nevertheless, that the arms in which the nervous connection is undisturbed (control) regenerates most rapidly.

As to the nature of this influence, it is evident from the study of the rate of general metabolism of half-disks with and without sense-organs that it is closely associated with if not identical with the control of the general metabolism of the animal. The latter experiments have not been carried far enough to give a definite answer to the question of whether or not there is a gradual decline in the difference in the rate of metabolism corresponding to that shown in regeneration.

This result clearly supports the general contention of Child, that the influence of the nervous system on regeneration is indirect rather than direct, but does not confirm his statement that there is a direct relationship between the rapidity of regeneration and the "characteristic motor activity of the parts concerned." It is shown, on the contrary, that motor activity may be greatly increased without altering to a proportional extent either the rate of regeneration or general metabolism.

The rate of regeneration appears to be simply one expression of the general metabolic activity of an organism and consequently to be subject to the control of the nerve-centers in the same manner as the many other functional activities—for some of which, at least, a direct nervous control can not be denied.

INFLUENCE OF THE SENSE-ORGANS ON THE LOSS IN WEIGHT DURING STARVATION.

The influence of the sense-organs on the rate of regeneration is so clearly marked when muscular activity is excluded as the determining factor that it seemed apparent that other metabolic activities might be used as a measure of this influence.

Mayer (1914) has shown that when *Cassiopea* is starved in sea-water from which all food material has been removed by either careful filtration or by heating to 71° C. and restoring by distilled water the amount lost by evaporation, the loss of weight follows a course which can be expressed mathematically by the formula $y=w(1-a)x$, in which y = the weight on any given day, w the original weight, x the number of days' starvation, and a constant, "the coefficient of negative metabolism." The greater part of the loss in weight appears to be borne by the mesoglœa, since this tissue constitutes by far the greater bulk of the body, as is shown by histological examination of the tissues and chemical analysis of medusæ before and after starvation. The simple character of the law which governs the loss of weight in *Cassiopea* during starvation also indicates that substances of the same sort serve to maintain the medusa throughout nearly the entire course of starvation. As the result of starvation the cells become reduced in size, some degenerate and disappear, while at the same time the mesoglœa becomes highly vacuolated. Within the short period of time covered by these experiments very little histological change could be recognized in the cells. The change in the appearance

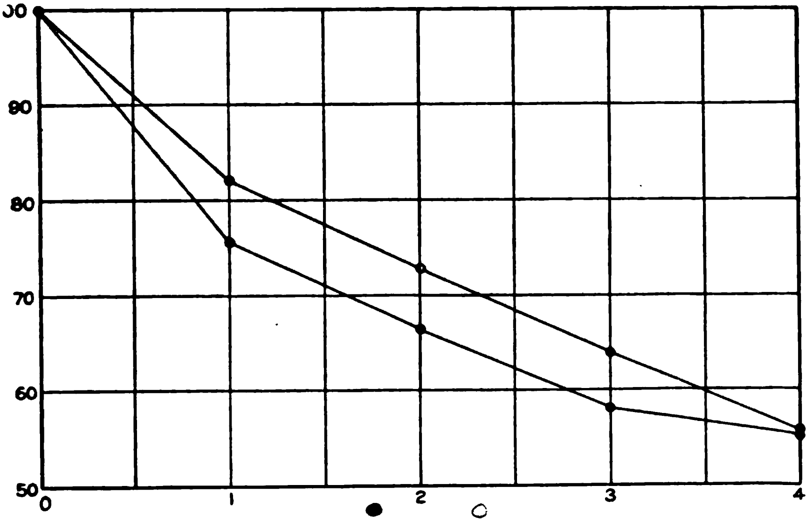


FIG. 10.—Showing loss in weight of active and inactive half-disks during starvation. (Compare table 9.) The divisions along the ordinate represent percentages of the original weight; those along the abscissa represent time in days.

of the mesoglœa was, however, much more marked in that vacuolization had become more easily noticeable.

There is clearly a different tissue chiefly involved in the loss of weight during starvation and in regeneration (see page 131), so that these experiments present a standard of measurement of metabolic activity sharply distinguished from that employed in the study of regeneration.

TABLE 6.—*Record for 0 to 4 days.*

[Twenty disks to show the differences in loss of weight during starvation between active and inactive half disks.]

Specimen.		0 day.	1 day.	2 days.	3 days.	4 days.
No. 1	Active	11	7.75	6.25	5.30	4.75
	Inactive	9.5	7	6.00	4.75	4.50
No. 2	Active	26	20	16.75	14	13
	Inactive	22	11	15	12.75	11
No. 3	Active	25	19	17	14.75	13.5
	Inactive	30	24.5	21.5	18	16.25
No. 4	Active	28	23	16.75	17.50	15.5
	Inactive	30	26	22	19.25	17.75
No. 5	Active	23	18	15	13.5	13.50
	Inactive	24	20.5	15.5	14.25	12.75
No. 6	Active	21.5	16.5	13.5	12.5	10.25
	Inactive	19.5	15.5	13.25	11.5	10.25
No. 7	Active	23.5	18.5	16	14	13
	Inactive	25	20.5	16.75	14.25	13
No. 8	Active	15	11.5	10	8.00	7.25
	Inactive	16.5	13.5	10.75	9.00	8.25
No. 9	Active	23	18	15	12.75	11.75
	Inactive	25	20.75	17.5	15	13.00
No. 10	Active	25	19.75	17	15	13.5
	Inactive	27	22	18.5	16.5	14
No. 11	Active	23	19.25	16	14	12.50
	Inactive	21	17.25	14.5	12.25	11.50
No. 12	Active	22	18	15	13.5	11.75
	Inactive	18	14.5	13.25	10.75	9.75
No. 13	Active	29.75	23.25	20.5	17.50	16.00
	Inactive	27	22.50	18.75	15.75	14.00
No. 14	Active	23	18	15.25	13.1	11.75
	Inactive	22	18.5	16.00	13.75	12.00
No. 15	Active	28	20.75	17.5	15	13.5
	Inactive	28	22.75	20	16.25	14.75
No. 16	Active	16	11.5	9.60	8.00	7.00
	Inactive	15	11.50	9.00	8.00	6.75
No. 17	Active	14	10	8.50	7.25	6.70
	Inactive	5	12.25	10.25	9.00	7.75
No. 18	Active	23.5	19.25	16.50	15	13.00
	Inactive	23	18.75	16	13.75	12.75
No. 19	Active	21	16	13.75	12.00	10.50
	Inactive	20.5	16.75	13	11.50	10.25
No. 20	Active	31	24.5	21.5	19.20	17.25
	Inactive	31	25.75	22.75	20.00	17.75

Mayer (*op. cit.*) has pointed out that the value of *a* (the coefficient of negative metabolism) has a different value for practically every set of experimental conditions. Thus this value differs when employing entire medusæ, regenerating portions of oral arms or the bell-margin, disks from which arms and stomach had been removed, or half-disks. Besides, on account of the relative activity of the zoöxanthellæ (symbiotic algæ), the rate of starvation differed greatly when the experiments were carried on in the diffuse light of the laboratory or in darkness.

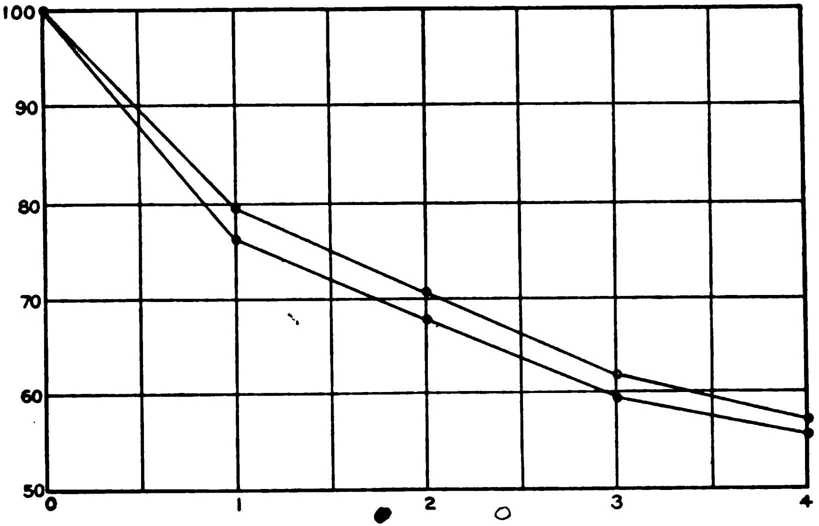
TABLE 7.—*Record for 0 to 4 days.*

[Twenty disks to show the differences in loss of weight between active and activated half-disks.]

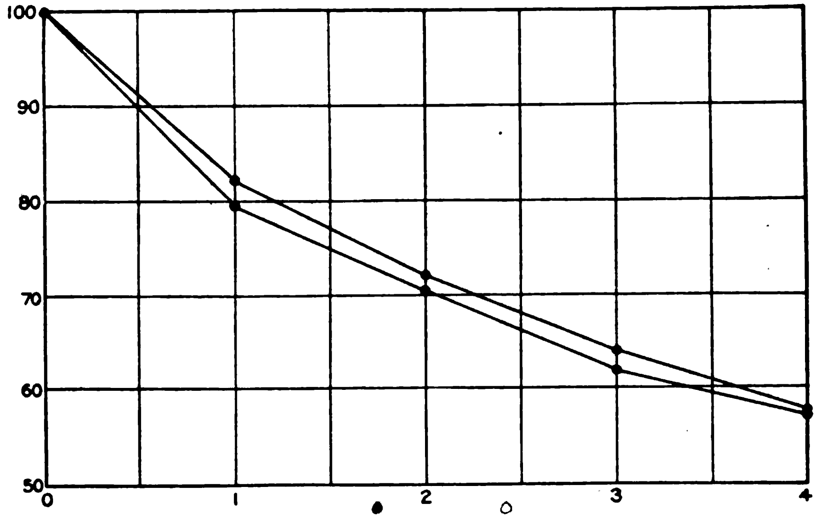
Specimen.		0 day.	1 day.	2 days.	3 days.	4 days.
No. 1	Active	28	20.5	18.00	16.25	14.50
	Activated	29	21	18.50	17.75	16.75
No. 2	Active	29.5	20.5	18.75	15.75	13.00
	Activated	29.25	21.5	20.25	17.50	15.25
No. 3	Active	31	22.5	21.00	19.25	17.50
	Activated	29	21.75	21.00	18.00	16.25
No. 4	Active	31.5	23.00	21.75	18	15.50
	Activated	29.5	23.00	21.75	18.5	16.25
No. 5	Active	29.5	21.00	18.75	18.00	17.25
	Activated	27.75	23.5	20.25	20.00	19.00
No. 6	Active	31.25	23.25	20.00	17.00	15.00
	Activated	31	24	22.50	18.50	15.25
No. 7	Active	20	20	18.75	16.50	14.75
	Activated	32.5	24.5	22.25	20.25	18.75
No. 8	Active	34.5	25.5	23.5	21.25	19.75
	Activated	34	27.5	24.5	22.50	20.75
No. 9	Active	34.5	18.00	16.25	14.50	13.00
	Activated	25.5	18.75	17.50	16.00	15.25
No. 10	Active	24	18.5	16.5	15.25	14.50
	Activated	24.25	19.25	18.00	16.00	14.75
No. 11	Active	24.25	19.25	18.00	15.50	13.25
	Activated	26.25	21.75	21.00	19.25	17.50
No. 12	Active	22.00	17.00	15.25	13.50	12.50
	Activated	22.25	16.50	15.50	14.50	13.75
No. 13	Active	28.5	23.25	21.50	19.25	17.75
	Activated	32.25	24.5	22.80	21.5	20.25
No. 14	Active	32.25	25.00	22.00	20.5	18.75
	Activated	20	24.00	21.75	20.25	19.00
No. 15	Active	34.25	27.25	25.00	22.75	20.25
	Activated	34	27.50	24.25	23.00	21.00
No. 16	Active	37.25	29.25	27.5	24.00	21.75
	Activated	35.75	27.50	24.50	23.25	21.25
No. 17	Active	21.00	15.50	13.50	12.50	10.75
	Activated	26.00	19.75	17.50	16.00	14.75
No. 18	Active	37.00	25.25	23.6	21.50	19.75
	Activated	31.00	25.50	24.5	21.50	20.00
No. 19	Active	21.24	17.50	15.75	13.50	11.25
	Activated	24.00	20.5	18.00	16.00	14.50
No. 20	Active	24.00	16	14.25	13.00	12.00
	Activated	24.00	16.25	16	16.00	15.50

In these experiments, where the disks were prepared by one of the three types of operations previously described, there was no necessity for taking precautions to remove the normal food materials from the water. Water from the regular laboratory supply pumped through vulcanite pipes by a vulcanite pump was regularly used, as no difference was found when water brought in glass jars from the ocean was used.

Mayer has called attention to the fact that regenerating specimens, during the first days of any experiment, starve more rapidly than normal medusæ.



9 FIG. 11.—Showing loss of weight of active and activated half-disks during starvation. (Compare table 10.) The divisions along the ordinate represent percentages of the original weight; those along the abscissa represent time in days.



9 FIG. 12.—Showing loss in weight of activated and inactive half-disks during starvation. (Compare table 11.) The divisions along the ordinate represent percentages of the original weight; those along the abscissa represent time in days.

In the same manner during the first half day of any experiment the member of any pair of half disks which showed the highest metabolic activity showed also the greatest loss in weight, yet even in the course of these experiments the difference in rate became less marked toward the end of the period of observation. This appears most clearly in experiments in which active and inactive specimens are compared (fig. 10), while the nearest approach to actual convergence is shown in the series in which activated and inactive specimens are compared (fig. 1).

TABLE 8.—*Record for 0 to 4 days.*

[Twenty disks to show the differences in loss of weight between activated and inactive half-disks.]

Specimen.		0 day.	1 day.	2 days.	3 days.	4 days.
No. 1	Activated	30.00	24.00	21.75	20.25	19.00
	Inactive	29.5	26.25	22.50	20.00	18.25
No. 2	Activated	22.25	16.50	15.50	16.50	13.75
	Inactive	22.75	19.00	16.25	14.00	12.50
No. 3	Activated	26.00	20	17.50	16.25	14.25
	Inactive	26.50	22.25	18.50	15.25	13.75
No. 4	Activated	28	23.75	20.50	18.75	17.00
	Inactive	29.5	25.75	21.75	19.00	16.75
No. 5	Activated	17.25	13.50	11.75	10.00	8.75
	Inactive	17.75	14.25	12.75	10.25	9.00
No. 6	Activated	33.5	27.25	24.50	22.00	20.00
	Inactive	35.00	29.00	25.50	22.25	19.50
No. 7	Activated	21.5	16.25	15.00	13.75	12.25
	Inactive	23.00	18.75	16.25	14.00	12.75
No. 8	Activated	23.5	16.00	15.25	14.50	13.00
	Inactive	24.00	20.75	16.25	14.50	12.25
No. 9	Activated	27.75	23.25	20.00	17.25	13.75
	Inactive	29.00	23.50	20.75	17.25	14.75
No. 10	Activated	31.00	24.75	23.25	20.75	17.75
	Inactive	31.50	26.75	22.75	20.50	17.25
No. 11	Activated	24.75	19.25	17.50	15.75	13.00
	Inactive	23.50	20.00	17.25	14.75	12.50
No. 12	Activated	21.50	16.75	15.00	13.25	11.75
	Inactive	22.00	17.75	15.00	13.25	11.50
No. 13	Activated	27.00	22.50	19.75	18.00	15.50
	Inactive	27.50	22.25	19.75	17.00	14.75
No. 14	Activated	23.75	18.00	16.50	14.25	12.00
	Inactive	25.00	20.25	17.50	15.25	12.75
No. 15	Activated	19.75	15.75	12.50	10.75	9.00
	Inactive	20.50	17.00	13.75	11.50	10.00
No. 16	Activated	26.25	20.25	17.75	15.25	13.00
	Inactive	27.00	22.75	19.00	15.75	13.50
No. 17	Activated	20.75	16.50	14.25	13.00	11.50
	Inactive	21.50	19.25	16.50	14.75	12.25
No. 18	Activated	18.50	15.00	13.25	12.50	10.00
	Inactive	20.00	16.00	13.75	11.75	10.25
No. 19	Activated	24.50	18.75	16.25	14.00	11.75
	Inactive	25.25	21.00	17.75	15.25	13.75
No. 20	Activated	27.00	21.25	19.00	16.25	13.50
	Inactive	28.00	23.00	19.75	16.25	13.75

The previous researches on inanition in invertebrates have dealt with either the chemical or histological changes brought about by the disturbances in normal metabolism caused by this condition, and need not be reviewed in this connection. In general, it has been observed that there is a shrinkage in size and frequent degeneration of the cells of all parts of the body. The epithelial tissues in general have a tendency to lose their cell-wall and to become a syncytium. Usually the loss of material is not nearly so selective as in the vertebrates; practically all organs become involved in the loss in an early stage of inanition, except in forms in which there is some special storage tissue, as the fat-bodies of insects.

The much more extensive literature dealing with inanition in vertebrates shows that the loss of body-substance takes place in a highly selective manner, involving many tissues before any marked loss is undergone by the "vital organs." Several researches in this field have demonstrated the importance of metabolic muscular activity as a factor in determining the rate of tissue destruction. The usual course of the changes involved in inanition is not, however, greatly altered by differences in the rate of metabolism, the one factor most influenced being the rate of occurrence of these changes.

TABLE 9.

Days after operation.	Loss of weight in active and inactive half-disks.		Loss of weight in active and activated half-disks.		Loss of weight of activated and inactive half-disks	
	Weight of half with sense-organs.	Weight of half without sense-organs.	Weight of half with sense-organs.	Weight of activated half.	Weight of activated half.	Weight of half without sense-organs.
0 day.....	100	100	100	100	100	100
1 day.....	75.34	81.81	76.29	79.41	79.47	81.97
2 days....	66.72	71.27	67.18	70.58	70.83	71.84
3 days....	58.54	64.09	59.76	61.99	62.03	63.91
4 days....	55.27	55.41	55.88	57.18	57.21	57.23

The results of three series of experiments, in each of which the halves of 20 disks were prepared in such a manner that they fell into one of the three categories described on pages 127 and 128, are presented in tables 6, 7, and 8, and figures 10, 11, and 12.

A summary of the results for each of the series of experiments is given in their proper order in table 9, in which the weights are reduced to terms of 100 grams for the original weight of each set of half-disks in each series. A comparison of these tables and the curves for the loss of weight with those given for the rates of regeneration under similar operations show a striking similarity in the results when those obtained by the use of these standards of measurement are compared, although, as previously stated, the tissues chiefly concerned in the two forms of metabolic activity are fundamentally different.

Table 10 gives the record of the halves of 10 disks prepared to give active and inactive specimens and shows loss in weight, decrease in diameter, measured for each half-disk at the line of separation of the halves, and shrinkage in the diameter of a cavity originally 22 mm. In several instances the diameters of the halves of the same disk vary 1 to 3 mm. on account of irregularities in the outline due to the cuts made in removing the sense-organs or the similar-shaped pieces of tissue between the sense-organs of the active half-disk.

The results of the measurements of size are completely in accord with those obtained in studying the loss in weight of the same half-disks, or the larger series of specimens prepared in the same manner, as shown in table 6. Although in the greater number of regeneration experiments no careful measurements were made of the decrease in the diameter of the cavity in which the amount of regeneration was measured, the differences in this regard between the different types of specimens was easily distinguishable in all cases and was always parallel to the relative rate of regeneration shown by either one of a given pair of half-disks, by whichever of the three operations they had been prepared.

Table 10.—*Record for 0 to 4 days.*

[Ten disks separated into active and activated halves, to show the comparative loss in weight, decrease in diameter, and decrease in diameter of the cavity where a disk of tissue had been removed in order to measure the amount of regeneration.]

Specimen.		0 day.			1 day.			2 days.			3 days.		
		Weight.	Diameter of disk.	Diameter of cavity.	Weight.	Diameter of disk.	Diameter of cavity.	Weight.	Diameter of disk.	Diameter of cavity.	Weight.	Diameter of disk.	Diameter of cavity.
No. 1	Active....	37	132	22	29	108	12	26	118	11	24.5	106	10.5
	Activated.	38			32	118	12	29	114	12	27	111	11.75
No. 2	Active....	18	110	22	13	83	11	11	75	9	10	68	8.25
	Activated.	21			16.5	99	14	14	89	11.5	12	80	11.00
No. 3	Active....	37	137	22	26	110	12	23	100	10	20.5	92	9.00
	Activated.	38			32	127	17	27	113	11	23.5	103	9.50
No. 4	Active....	31	130	22	22.5	98	13	21.5	93	9	18	87	8.00
	Activated.	31			26.5	118	17	23	110	11	20.5	104	9.75
No. 5	Active....	24	118	22	18	96	11	16	86	10	14.00	78	8.50
	Activated.	24			18	110	19	16	94	12	14.00	85	10.75
No. 6	Active....	20	110	22	14	86	13	12	79	10	10.00	71	8.75
	Activated.	20			15.5	100	16	14.25	92	13	11.5	81	11.50
No. 7	Active....	28	125	22	21.5	109	13	19	98	10	16	89	8.00
	Activated.	27.5			23	117	16	20	105	12	18	99	9.50
No. 8	Active....	28	123	22	21	93	12	19	88	10	16	84	8.25
	Activated.	30			25	118	17	21.5	112.5	12	19	107	10.50
No. 9	Active....	28	122	22	22.5	110	11	19.5	95	11	16	86	10.50
	Activated.	25			20	110	15	17	97	12	14.5	90	10.75
No. 10	Active....	28	125	22	22	112	13	19	99	11	16.5	91	9.75
	Activated	29			26	120	18	23	107	13	19.5	98	11.25

INFLUENCE OF SENSE-ORGANS ON THE RATE OF GENERAL METABOLISM AS MEASURED BY CO_2 PRODUCTION.

The results of the experiments recorded in the two preceding sections show that the nerve-centers exert a decided influence on the rate of metabolic activities when measured by two different standards. It seems apparent also that, although the standards of measurement adopted—regeneration and loss of weight during starvation—largely involve the activities of distinct tissues, the influence of the sense-organs is exerted in a manner strikingly similar in the two cases. This similarity in result suggests the probability that the effects measured are only different phases of the general metabolic activity of the organism, which when measured as a whole would show an influence of the nerve-centers comparable to that obtained when the activities involved in distinct functions are used as the unit of measurement.

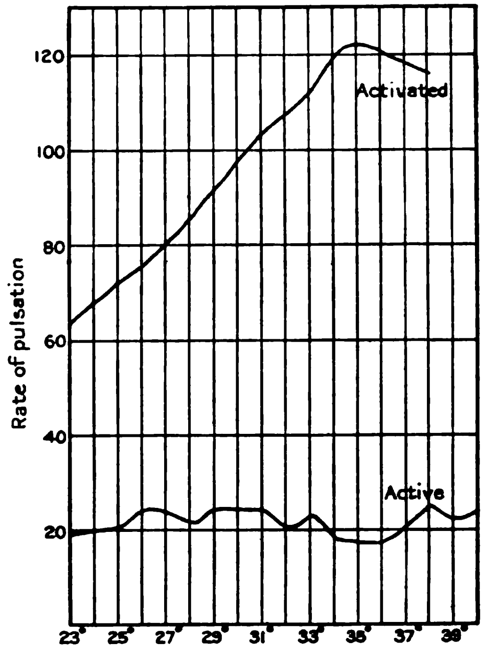


Fig. 13.—Showing effect on rate of pulsation of active and activated halves of the same medusa disk when subjected to an increase in temperature from 23° to 40° C. The upper curve is for the activated specimen; the lower curve for the active specimen.

The measurement of the amount of carbon dioxide given off by a half-disk within a given space of time affords means of determining the comparative rate of total metabolic activity of specimens under the operative conditions used in the previous experiments. The technique of the experiments was as follows: The half-disks were prepared in the manner previously described, so that active, activated, and inactive individuals were secured for comparison. Each half-disk of any pair was placed in a separate jar containing about 1,200 c.c. of fresh sea-water brought from the ocean at the time of beginning a set of experiments. The capacity of the jars varied slightly and pairs of equal volume had to be selected for the two halves of the same disk.

As the measurable amount of CO_2 produced and consequently the rate of pulsation for some time after the beginning of an experiment were largely dependent upon the activity of the zooxanthellæ, most of the experiments were started late in the afternoon and therefore continued during the hours of darkness. When experiments were going on during the day the necessity for covering the jars with dark cloth caused a decided rise in temperature, which increased unnecessarily the complication of the calculations involved in determining the amount of CO_2 given off in respiration.

Preliminary experiments along this line were undertaken, in collaboration with Dr. S. Tashiro, in 1915, when the determinations were made by means of titrating the sea-water with n/100 NaOH, using phenolphthalein as an indicator and titrating to the color obtained by adding the same amount of indicator to an equal volume of sea-water taken as a control at the beginning of each experiment.

In the later experiments the amount of CO_2 given off by each half-disk was determined by ascertaining the P_H (minus log hydrogen-ion concentration) of a sample of sea-water taken as a control at the beginning of the experiment, and that from the jar in which the specimen had been respiring during the experiment. Both specimens involved in an experiment were removed from the jars at the same time, whenever the determinations were to be made or either specimen had ceased pulsating. The volume of CO_2 necessary to bring about a corresponding change in the hydrogen-ion concentration of an equal volume of sea-water (1,200 c.c.) was determined by adding known volumes of CO_2 to that volume of sea-water.

The results of these determinations are shown in figure 13. This result confirms McClendon, who shows that the CO_2 content of sea-water can be more accurately measured by determining the hydrogen-ion concentration than by a direct determination of the CO_2.

When the half-disks were placed in the closed jars the increase in the hydrogen-ion concentration of the sea-water caused at first an increase in their rate of pulsation. This was followed after 3 or 4 hours by a gradual decline in rate until finally both activated and active half-disks had been completely narcotized by the CO_2 which they had themselves given off. The decline of the rate of activated half-disks was very regular and the pulsations became gradually weaker, until scarcely discernible before entirely ceasing. The active specimens, on the contrary, behaved very erratically. Frequently when the pulsation had become very slow, or had even ceased for several minutes, these specimens would suddenly take up rapid pulsation and for a short time their activity would be even greater than when under the influence of the first stimulating effect of the increased hydrogen-ion concentration in the early part of the experiment.

The activated half-disk was invariably the first to become narcotized, the muscles in these, as in all other experiments involving anesthetics, being less resistant to such agents than the sense-organs.

The activated specimens, when once their pulsations had ceased, were incapable of again taking up pulsation unless stimulated to activity by artificial means. The active specimens, even after having been quiescent for several hours, would take up pulsation within 2 minutes after being put into fresh sea-water.

When such experiments were carried on in daylight narcosis would not be produced during the first day, because of the reduction of the amount of CO_2 in the water by the photosynthetic activity of the zooxanthellæ.

The disks were quickly narcotized on being put into sea-water to which had been added enough CO_2 to change the hydrogen-ion concentration to that shown by the water in the jars in which the half-disks had ceased pulsating. It seems apparent, therefore, that narcosis had been caused entirely by the increase in CO_2 and not by accumulation of other waste products of metabolism, or by a decrease in the amount of oxygen available for respiration.

Table 11 shows the results of a typical experiment for determining the total metabolism of active and activated halves of the same disk. The hydrogen-ion concentration is given in terms of the P_H numbers of Sorensen. The sea-water at the beginning of the experiment had a hydrogen-ion concentration of $P_H = 8.1$ ($C_H = 0.793 \times 10^{-8}$).

TABLE 11.

Specimen.	Weight.	Pulsation-rate.			P_H.
		$1^h\,45^m$ p. m.	$4^h\,40^m$ p. m.	$7^h\,30^m$ p. m.	
	grams.				
No. 1, *a*.....	22.8	44	62	18	7.80
No. 1, *b*.....	23.0	128	136	120	7.90
No. 2, *a*.....	30.0	36	34	22	8.00
No. 2, *b*.....	30.5	88	86	98	8.00
No. 3, *a*.....	39.0	44	36	8	7.90
No. 3, *b*.....	41	130	158	126	7.90
No. 4, *a*.....	28	56	22	25	7.90
No. 4, *b*.....	29	96	106	Out.	8.00
No. 5, *a*.....	29.25	84	32	32	7.90
No. 5, *b*.....	28.75	116	120	132	7.90

These experiments were not carried out in sufficient detail to determine the regular course of respiration or the normal respiratory quotient under the influence of varying hydrogen-ion concentration, their aim being to determine the influence of the sense-organs on the rate of total metabolism.

The differences due to muscular activity conform to those obtained from the measurements of rate of regeneration and loss of weight during starvation. The amount of respiration of an active half-disk was invariably as great as its activated mate in spite of the great differences in muscular activity. Indeed, in only a very few instances did the normal active specimen show as low a CO_2 production as the more rapidly pulsating activated half of the same disk.

The activated half-disks were narcotized when the hydrogen-ion concentration of the sea-water in which they were contained had reached about 0.126×10^{-7} ($P_H = 7.9$). In fact, a greater concentration of hydrogen ions was seldom reached in the water in which the activated disks were kept, as after the animals had been sufficiently narcotized to cause the cessation of pulsation other metabolic activities were also interfered with to such an extent that respiration practically ceased. Active half-disks were capable of maintaining at least intermittent pulsation until the hydrogen-ion concentration had become 0.159×10^{-7} ($P_H = 7.8$). Nearly all the determinations of the H concentration were made by means of color comparison with tubes of known H concentration prepared by Hynson Westcott & Dunning, from buffer mixtures standardized by J. F. McClendon, containing mixtures of phosphates and borates using thymolsulfonephthalein for concentrations above $P_H = 8$ and phenolsulfonephthalein for concentrations below $P_H = 8$. These tubes were in no part of their range graduated more closely than $P_H = 0.1$, so that no very fine readings could be obtained except by estimating fractions of this unit, which could not be done with any considerable degree of accuracy.

INFLUENCE OF SENSE-ORGANS ON THE CHANGE IN RATE OF PULSATION IN RESPONSE TO CHANGES IN TEMPERATURE.

The studies of Harvey (1911) and Mayer (1913) have shown that when *Cassiopea* is slowly heated the rate of nerve-conduction increases from about 18° C. to about 36° C., after which there is a rapid decline in the rate. Recovery of the medusa is impossible if the temperature is carried much above 40° C.; indeed, when cooling is begun before a fatal temperature is reached the normal rate of pulsation does not return even when the medusa has been kept at normal temperature for several days. In its general features the curve for the rate of nerve-conduction in *Cassiopea* resembles that for enzyme action, as was pointed out by Harvey, and as shown for the pulsation-rate of the vertebrate heart (Knowlton and Starling (1912) *et al.*).

Since Mayer (1908) has shown that the initiation of the stimulus for pulsation takes place in the sense-organs through a definite chemical reaction, it was thought possible that if a sense-organ were subjected to changes in temperature while the body of the disk was kept at a

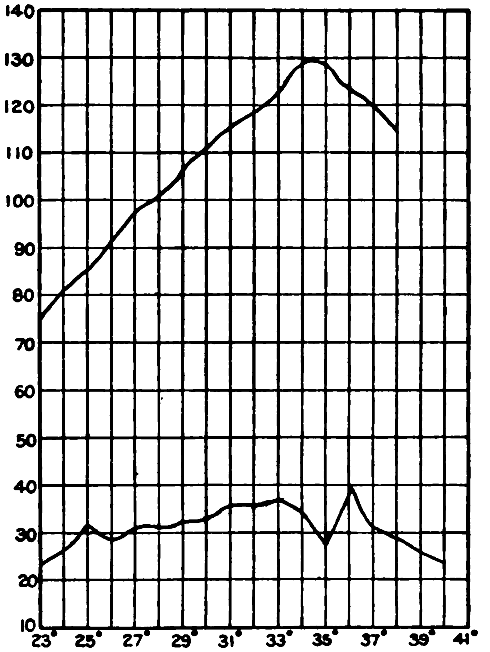


FIG. 14.—Curves showing averages of all determinations of effect of change in temperature on active and activated halves of the same disk.

constant temperature, the resulting rate of pulsation might follow Van't Hoff's law more closely than when the entire disks were subjected to the change in temperature, or the rate of pulsation—nerve-conduction—of activated disks under similar conditions was measured. Such experiments showed, however, that the results from this cause were in no essential different when either a single sense-organ, an "active" disk, or activated disk were subjected to the changes in temperature. All my later experiments were therefore confined to those in which active and activated halves of the same medusa disk were subjected to the temperature changes at the same time in order to determine the influence of sense-organs upon the response to changes in temperature. The prepared half-disks were placed in a 4-liter jar of fresh sea-water which was in turn contained in a 20-liter jar of fresh water supported on a copper tray filled with water. The water surrounding the inner jar was cooled by the addition of ice or heated by the flame of a small alcohol lamp so that a change of 1° in the temperature of the sea-water was obtained in 15 minutes. The activity of the medusæ was sufficient to agitate the water to such an extent that at no time could a difference of 0.1° C. be detected in the temperature of any two portions of the contents of the jar.

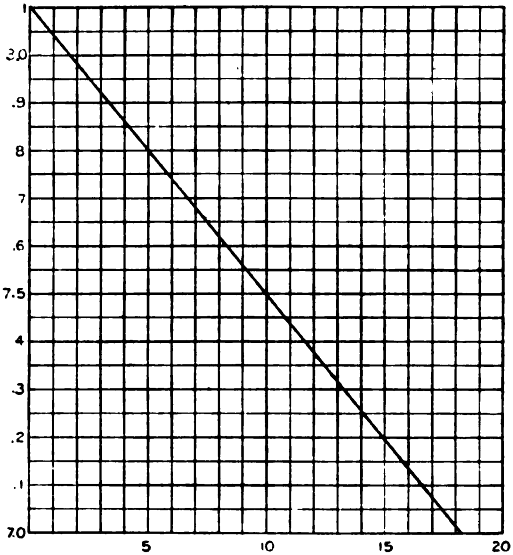


FIG. 15.—Showing change in hydrogen-ion concentration when a known volume of carbon dioxide is added to 1,200 c.c. of Tortugas sea-water.

In beginning any experiment the temperature was lowered to the desired starting-point and then raised until either the desired upper limit was reached or continued until the disks became inactive. On being cooled below 20° C. the activated specimens often ceased pulsating and could not again be aroused to steady pulsation by the application of repeated stimuli until the temperature had been raised to about 22° C. From 23° to 33° C. the increase in rate of pulsation for activated specimens followed a right line (fig. 14) and within that limit the rate was nearly doubled. The active half-disks, over the same range of temperature, gave generally a smaller increase in rate and the change in rate was always more erratic than that shown by those deprived of their sense-organs. (Fig. 14.)

The pulsation-rate of active specimens frequently reached its maximum at from 27° to 29° C. In other specimens the maximum rate was not attained until a temperature of 37° C. had been reached, after which the decline in rate was precipitous, death occurring at 38.5°.

A summary of all the results is given in figure 15, which shows the average for 11 active and 9 activated half-disks.

RELATION BETWEEN AREA OF TISSUE ENERVATED BY A SINGLE SENSE-ORGAN AND RATE OF PULSATION.

Eimer (1874) made the observation that as the area of tissue enervated by a single sense-organ was progressively decreased there was a corresponding decline in the rate of pulsation. He states that the decline in rate is directly proportional to the decrease in area through the range of the experiment.

Romanes (1895) repeated these observations and denied the conclusion of Eimer that the decline in rate was directly proportional to the decline in area; but he did not give any definite statement of the mathematical relation between area and rate of pulsation. Mayer (1906) confirmed and extended these latter observations, but again did not accurately determine the relation between tissue area and rate of pulsation.

In my own experiments all sense-organs but one were removed from each disk, and after allowing a period of 1 hour for recovery from the shock of the operation the rate of pulsation was recorded. Immediately after this record was completed the area under the control of the sense-organ was reduced to one-half its former extent by making a cut through the subumbrella tissues. In this manner the area of tissue was reduced to $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, and $\frac{1}{16}$ of that of the entire disk (fig. 16) and a record of the pulsation-rate was secured for each area. In all, 141 disks were used in this series of experiments.

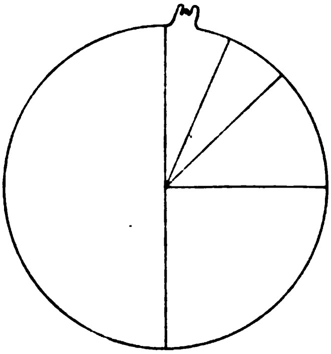


FIG. 16.—Showing method of operation employed in studying the relation between area of tissue innervated by a single sense-organ and the rate of pulsation.

A summary of the results is given in table 14 and figure 17.

As will be seen from the graph, the decline in rate follows a right line very closely, except at the point where the rate for $\frac{1}{8}$ the original area is shown. When extended to included areas of tissue less than $\frac{1}{16}$ of the original the decline in the pulsation-rate follows practically the same formula as shown in figure 17, by the broken line extending to a point where the tissue area under the control of the single sense-organ is reduced to $\frac{1}{64}$ the original size.

A reduction of the area of a disk beyond $\frac{1}{16}$ of the original could not be made accurately by the operation illustrated in figure 17.

When further reduction in area was desired, linear strips of constant width, each with a single sense-organ, were cut from the body of large medusæ, so that the area could be accurately reduced to $\frac{1}{32}$, $\frac{1}{64}$, $\frac{1}{128}$ and $\frac{1}{256}$ that of the original area. The fall in rate, as recorded for the few observations made, when graphically represented produced a continuation of the straight line obtained from the larger subdivisions of the disks.

The elimination of any portion from the excitable area could be produced by immersing that portion in a 0.4 m $MgCl_2$ solution, as shown in figure 18. When the anesthetizing solution was contained in the middle vessel the nerves were capable of transmitting the impulse across this area, where the muscles were inactive, to the tissue in dish No. 3, so that the inactive area could be obtained at any point in the strip of tissue. No differences in the results were obtained when the activity of a given area of muscles was eliminated by the cutting of the subumbrella tissues or their immersion in the $MgCl_2$ solution. Pieces of tissue in sea-water were found to be capable of responding to each separate induction shock when applied at the rate of 126 per minute when they had been reduced to $\frac{1}{512}$ of the original area of the strip which represented scarcely more than $\frac{1}{10000}$ ($\frac{1}{9817.48}$) of the area of the original disk.

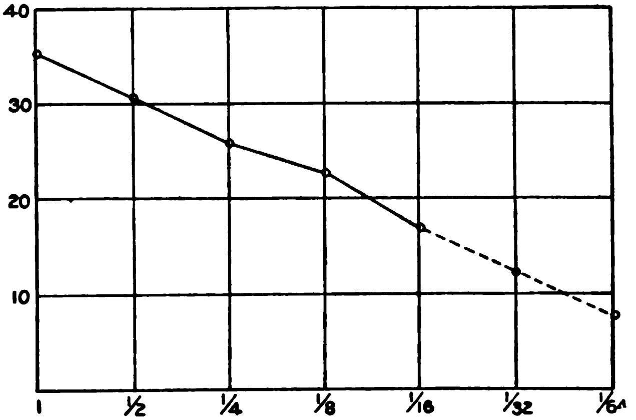


FIG. 17.—Showing graphically the results obtained from the study of 140 disks when the area of tissue was successively reduced to $\frac{1}{16}$ the original area. The broken line extending to an area $\frac{1}{64}$ the original is based on a smaller number of observations. The divisions along the ordinate represent the number of pulsations; those along the abscissa represent the area of tissue under the influence of the sense-organ.

This fact makes it apparent that the decline in rate of pulsation caused by reducing the area enervated by a single sense-organ is not at all dependent upon the necessity for a latent period on the part of the muscular tissues. On the other hand, there is no apparent reason why the sense-organs should be incapable of carrying on at the usual rate the chemical reaction by which sodium is liberated, unless they are dependent upon the other tissues of the subumbrella for some substance which in the smaller areas becomes reduced to such an extent that it can no longer be produced rapidly enough to allow the sense-organ to discharge at the usual rate.

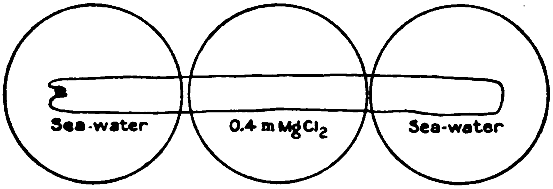


FIG. 18.—Showing arrangement of vessels by which any area of tissue could be rendered inactive in order to determine whether or not the method by which the area of muscle under the influence of a single sense-organ, was reduced was an important factor in determining rate of pulsation.

The fact that in a normal medusa the initiation of pulsation is taken up by one after another of the sense-organs at irregular intervals may indicate that, even while in normal relationship to the surrounding tissues, each sense-organ during its period of control of the pulsation of the medusa exhausts from its immediate surroundings some substance necessary for the maintenance of the discharge of impulses at a regular high rate. Since the pulsation-rate of a medusa is under normal conditions constant, each sense-organ must give up its control as soon as its power of giving out impulses falls below that necessary to maintain the usual rate of pulsation. When a given sense-organ has surrendered to a more active neighbor the initiation of pulsation, it will have a sufficient latent period to recover the chemical equilibrium necessary for bringing its rate of discharge of impulses up to that necessary to supplant any other center as the controlling factor in pulsation.

GENERAL DISCUSSION.

THE RELATION OF MUSCULAR ACTIVITY TO THE RATE OF METABOLIC PROCESSES IN CASSIOPEA.

(1) The relation of muscular activity to the rate of regeneration has been considered in the section dealing with the regeneration experiments and will be here taken up only in making comparison with the results obtained by other methods. It appears to be clearly established that in this organism muscular activity plays a relatively unimportant part in controlling the rate of metabolism as measured by the total CO_2 output or by either of the two other standards of measurement employed. The relatively small difference between activated and inactive specimens as measured by either of the three standards may be accounted for on the ground of differences in temperature induced by the extreme activity of the muscular system. While no estimate of the amount of heat generated through muscular activity is available for *Cassiopea*, the evidence secured from the study of the change in rate of nerve-conduction (pulsation) indicates that a relatively small temperature increase would be sufficient to account for the observed differences. In spite of this temperature effect, activated disks, pulsating at from 3 to 10 times the rate of active specimens, show metabolic activities (by whichever of our three standards measured) considerably lower than do the other halves of the same original disk, the activities of which are under the control of the sense-organs.

(2) With the elimination of muscular activity as an important determining factor in metabolic activity there remains to be considered the nature of the nervous control over metabolic activity. After 1851 (when Ludwig proved the existence of nerves producing activity of the cells of secreting glands) it was held for some time that there were nerve-fibers presiding over other metabolic activities, as growth and repair. With the increasing knowledge of the part played by chemical excitation in controlling many activities, the conception of the trophic function of nerves, in the broader sense, has been discarded by most modern students of physiology.

The trophic function in the special sense of Heidenhain (1858) has been clearly demonstrated by the researches of Babkin (1913) for the nerves of cranial origin to certain gland-cells.

Most studies on the trophic effect of nerves on growth or other general metabolic phenomena have given negative results. Jacobson (1910) concluded, from experiments on pigeons and dogs, that the denervated areas healed as rapidly as those with the normal nerve-supply. Tschermak (1910), after extended researches and a critical review of the literature on trophic and tonic innervation, states that there is no evidence for the existence of separate or specific trophic nerves. He considers it probable, however, that motor and sensory nerve-fibers convey trophic impulses as an accessory function, which would imply the necessity of qualitative differences in the impulses which traverse any fiber. On the other hand, the work of Head and Campbell, on *Herpes zoster*, supports the conception of a trophic function of nerve-fibers. In this disease the presence of change in the dorsal root ganglia causes abnormal impulses to be sent in an efferent direction along the sensory fibers of the skin. On account of this excitation, blistering of the skin takes place over the area of distribution of the fibers (axones) terminating in the affected ganglia.

Jacobson (*loc. cit.*) states that "in the lower animals the maintenance of life processes are largely a phenomenon of chemical coordination," and that trophic influences ought to be greater in the higher than in the lower animal phyla. Her own experiments showed, in her opinion, that there was no difference in this respect between pigeons and dogs, both animals giving negative results.

Among the lower invertebrates, in *Cassiopea* (and in this respect it is unlikely to differ markedly from other scyphomedusæ) the general metabolic activities, including regeneration (a form of growth), total CO_2 production, and the using of body-substances for metabolism when no food is being taken, are strongly influenced by the condition of presence or absence of some sort of impulses from the sense-organs (nerve-centers). That there are neurones which are specific conductors of trophic impulses as distinct from motor or sensory nerves is by no means implied by this statement. In fact, in a nervous system such as that of the medusæ it is not always possible, on morphological grounds alone, to separate those neurones which are sensory from those of a motor function. In the absence of even this means of establishing the identity of the character of a portion of the neurones it is useless to attempt, in the present state of our knowledge of the anatomy of this nervous system, to make any distinction between the character of the function of any particular portion of it.

In spite of this lack of completeness in our knowledge of the nature of the nervous system of *Cassiopea*, it should be emphasized that, through whatever morphological mechanism it may be exercised, the trophic influence of the sense-organs is a constant factor in the normal metabolic activity. Without these impulses, even while muscular activity is far above normal, all the measurable metabolic activities except that of the motor system are maintained at a considerably lower level than when under the control of the sense-organs, by which muscular activity is held at a much lower rate than that at which these tissues are capable of maintaining a constant activity for long periods.

SUMMARY.

(1) The experiments with entire disks, when the rates of regeneration of specimens on which the sense-organs remained are compared with those of specimens from which all sense-organs are removed, are inconclusive because of wide differences in physiological activity between different individuals.

(2) When we compare the insulated halves of a disk, on one of which the sense-organs remain, while all of them have been removed from the other half, it is found that the half-disk with sense-organs always regenerates most rapidly. This is especially noticeable in the early stages of regeneration. The difference in rate falls gradually throughout the course of an experiment (table 1 and fig. 6).

(3) When disks prepared as in the experiments mentioned in the previous paragraph are allowed to regenerate in sea-water plus 15 parts 0.6 m $MgSO_4$, the regeneration is at first more rapid from the half on which the sense-organs come under the influence of the anesthetic, and from that time on the rate of regeneration is practically equal from both halves (table 2 and fig. 7).

(4) When all the sense-organs are removed from a disk and the halves insulated, muscular activity may be maintained in one half by forming an endless labyrinth of the subumbrella tissue and initiating a circuit wave of contraction by induction shocks. Under these conditions the regeneration is faster from the activated than from the inactive half-disk. The difference is, however, not nearly so great as when the sense-organs are removed from only one of the insulated halves of a disk (table 3 and fig. 8).

(5) Comparison of rates of regeneration of the halves of a disk, one half of which retains its sense-organs, while a circuit wave of contraction is maintained in the muscles of the other half, shows that the half-disk the muscles of which are contracting under the control of the sense-organs regenerates faster, although the rate of pulsation of the activated half is more than 3 times that of the former (table 4 and fig. 9).

(6) The influence of the nervous system on the earlier stages of regeneration has been noted by several earlier investigators, but apparently no importance has been attached to it.

(7) These experiments indicate that the rate of regeneration is simply one expression of the general metabolic activity of an animal, and as such is subject to the influence of the nerve-centers, as are many other functional activities.

(8) When a normal medusa, a regenerating medusa, a medusa disk, or half-disk is kept in water free from food material, the loss in weight follows a curve of the formula $y = w(1-a)x$. Here y = the weight for any given day, w = original weight, x = number of days of starvation, and a is a constant, the "coefficient of negative metabolism." The value

of *a* will differ under different experimental conditions, but all observed instances agree closely with the expectations.

(9) The greater part of the loss in weight appears to be shown by the mesoglœa, which undergoes marked histological changes during the course of starvation.

(10) As in regeneration experiments, the greatest metabolic activity is shown by the active specimens, the least by the inactive, while the activated individuals stand in an intermediate position, being closest to the one in which muscular activity is entirely suppressed. (Table 9, and figures 10, 11, and 12.)

(11) Specimens as prepared for the previous experiments were kept for various periods in closed jars of sea-water, each containing about 1,200 c.c. The amount of CO_2 given off was ascertained by determining the hydrogen ion concentration of this water at the beginning and end of each experiment. The volume of CO_2 necessary to bring about the observed change was determined by adding known volumes of this gas to 1,200 c.c. of sea-water, as shown in figure 13.

(12) While the rate of pulsation of the activated half-disks was generally more than 3 times that of the active half of the same disk, the amount of CO_2 given off in respiration from the activated specimen was never more, and almost always considerably less, than that from the active specimens of any pair of half-disks. In general the results from this type of experiment are in perfect accord with those obtained from those recorded in sections I and II, where the standard of measurement was a component part of that used in the respiration experiments.

(13) The change in rate of nerve-conduction (pulsation) in activated specimens over a range in temperature from 23° to 33° C. follows nearly a straight line, the actual rate being approximately doubled for this increase of 10° C. Beyond 35° the rate rapidly declines, death ensuing at 38°. The curve for this change resembles very strikingly that for enzyme action. The change in rate of active half-disks is much more erratic. Such specimens can, however, be carried to much lower temperatures (14° C.) before activity ceases, and the upper limit of temperature was always higher by 2 or 3 degrees than for the activated half of the same disk. When a single sense-organ was subjected to changes in temperature, the remainder of the disk, without its sense-organs, being maintained at a constant temperature, the results showed no characteristic difference from those obtained when the whole disk was subjected to the temperature change.

(14) As the area of tissue enervated by a single sense-organ is successively reduced to one-half its former extent at each operation, the rate of pulsation declines until, when the area has been reduced to $\frac{1}{16}$ its original extent the pulsations will have been reduced to approximately half their original number. Beyond this point the reduction in rate of pulsation followed the same ratio to the area, at least until the final

area of $\frac{1}{128}$ its original extent. A piece of subumbrella tissue 0.0001 the original area of the disk is still capable of responding to each induction shock when applied at the rate of 126 per minute. Apparently, therefore, the necessity for a latent period on the part of the muscles is not an important factor in the decline in rate of pulsation. It appears more probable (as indicated by the manner in which each of the 16 sense-organs, usually present on a medusa, take up in turn the initiation of the stimulus for pulsation) that some chemical interchange between the sense-organs and the surrounding tissue is necessary in order that the activity of these structures shall be maintained at the highest state of efficiency. As the tissue area is reduced this readjustment takes place at a correspondingly lower rate, because the material essential for this adjustment is less readily available to the sense-organs.

(15) The evidence from the foregoing experiments establishes the fact that some sort of trophic influence is exerted in general metabolic activities by the sense-organs. The structure of the nervous system of *Cassiopea* makes it impossible, however, to prove the existence of trophic nerve-fibers as distinct from those of sensory or motor function. On the other hand, it is shown that in this lowly organized form there are transmitted from the nerve-centers certain influences that are absolutely essential for maintaining general metabolic activities at their normal rate.

LITERATURE CITED.

Babkin, B. P. 1913. Sekretorische und vasomotorische Erscheinungen in den Spehiceldrüsen. Pflügers Archiv., Bd. 149.

Bardeen, C. R. 1901. On the physiology of *Planaria maculata*, especially the phenomena of regeneration. Amer. Jour. Physiol., vol. 5.

———. 1903. Factors in heteromorphosis in planarians. Arch. Entw. Mech., Bd. 16.

Barfurth, D. 1894. Die experimentelle Regeneration überschüssiger Gliedmassentheile bei den Amphibien. Arch. Entw. Mech., Bd. 1.

Barnes, C. T. 1904. Internal factors of regeneration in *Alpheus*. Biol. Bull., vol. 5.

Cary, L. R. 1915. Studies on regeneration: The influence of the sense-organs on the rate of regeneration in *Cassiopea xamachana*. Carnegie Inst. Wash. Year Book No. 13.

———. 1915*a*. The influence of the marginal sense-organs on functional activity in *Cassiopea xamachana*. Proc. Nat. Acad. Sci., vol. 1.

———. 1916. The influence of the marginal sense-organs on the rate of regeneration in *Cassiopea xamachana*. Jour. Exper. Zool., vol. 21, No. 1.

———. 1916*a*. The influence of the marginal sense-organs on metabolic activity in *Cassiopea xamachana* Bigelow. Proc. Nat. Acad. Sci., vol. 2, No. 12.

Child, C. M. 1904. Studies on regulation, V. The relation between the central nervous system and regeneration in *Leptoplana*. Jour. Exper. Zool., vol. 1.

———. 1904*a*. Studies on regulation, VI. The relation between the central nervous system and regeneration in *Leptoplana*. Anterior and lateral regeneration. Jour. Exper. Zool., vol. 1.

———. 1905. Studies on regulation, IX. The positions and proportions of parts during regulation in *Cestoplana* in the presence of the cephalic ganglia. Arch. Entw. Mech., Bd. 20.

———. 1905*a*. Studies on regulation, X. The position and proportion of parts during regulation in *Cestoplana* in the absence of the cephalic ganglia. Arch. Entw. Mech., Bd. 20.

———. 1910. The central nervous system as a factor in the regeneration of polyclad turbellaria. Biol. Bull., vol. 19.

Eimer, **Th.** 1874. Ueber künstliche Theilbarkeit von Aurelia aurita, etc. Verhandl. Physik.-Med. Gesellschaft Würzburg, N. F., Bd. 6.

Godlewski, E. 1905. Versuche über den Einfluss des Nervensystems auf die Regenerationserscheinungen der Molche. Bull. Inter. Acad., Cravoie.

Goldstein, K. 1904. Kritsche und experimentelle Beiträge sur Frage nach dem Einfluss des Zentralnervensystems auf die embryonale Entwicklung und die Regeneration. Arch. Entw. Mech., Bd. 18.

Goldfarb, A. J. 1909. The influence of the nervous system in regeneration. Jour. Exper. Zool., vol. 7.

———. 1912. The central nervous system in its relation to the phenomena of regeneration. Arch. Entw. Mech., Bd. 32.

Harvey, E. N. 1911. The effect of different temperatures on the medusa *Cassiopea* with special reference to the rate of conduction of the nerve impulse. Carnegie Inst. Wash. Pub. No. 132.

Head, H., and W. A. **Campbell**. 1900. The pathology of *Herpes zoster* and its bearing on sensory localisation. Brain, vol. 23.

Heidenhain, R. 1868. Beiträge sur Lehre von der Speichel absonderung. Studien Physiol. Inst., Breslau, No. 4.

Herbst, C. 1896. Ueber die Regeneration von antennenähnlichen Organen an Stelle von Augen. Arch. Entw. Mech., Bd. 2.

———. 1899. Ueber die Regeneration von antennenähnlichen Organen an Stelle von Augen. Arch. Entw. Mech., Bd. 9.

Hines, C. S. The influence of the nervous system on the regeneration of the leg of *Diemyctylus*. Biol. Bull., vol. 10.

Jacobson, **Clara**. 1910. The rate of healing of wounds in denervated skin areas and its bearing on the theory of trophic nerves. Amer. Jour. Physiol., vol. 26.

KNOWLTON, F. P., and E. H. STARLING. 1912. The influence of variations in temperature and blood-pressure on the performance of the isolated mammalian heart. Jour. Physiol., vol. XLIV.

LILLIE, F. R. 1901. Notes on regeneration and regulation in planarians. Amer. Jour. Physiol., vol. 6.

LUDWIG, CARL. 1851. Neue Versuche über die Beihülfe der Nerven zu der Speichelsecretion. Mitt., No. 50, Zürich Naturforsch. Ges. Zs. rat. Med. N. F. 1.

MAYER, A. G. 1906. On rhythmical pulsation in Scyphomedusæ. I. Carnegie Inst. Wash. Pub. No. 47.

———. 1908. On rhythmical pulsation in Scyphomedusæ. II. Carnegie Inst. Wash. Pub. No. 102.

———. 1914. The effect of temperature upon trophical marine animals. Carnegie Inst. Wash. Pub. No. 183.

———. 1914*a*. The law governing the loss of weight in starving *Cassiopea*. Carnegie Inst. Wash. Pub. No. 183.

MORGULIS, S. 1908. Regeneration in the brittle star *Ophiocoma*, with especial reference to the influence of the nervous system. Proc. Amer. Acad. Arts and Sci., vol. 44.

———. 1912. Beiträge zur Regenerationsphysiologie. VI. Ueber das Verhältnis des Nervensystems zur Regeneration. Arch. ges. Phys., Bd. 143.

MORGAN, T. H. 1901. Regeneration in the egg embryo and adult. Amer. Nat., vol. 35.

———. 1902. Experimental studies of the internal factors of regeneration in the earthworm. Arch. Entw. Mech., Bd. 14.

———. 1902*a*. The internal influences that determine the relative sizes of double structures in planarians. Biol. Bull., vol. 3.

———. 1904. Notes on regeneration. Biol. Bull., vol. 6.

NUSBAUM, J. 1908. Beitrag zur Frage über die Abhängigkeit der Regeneration vom Nervensystem bei Nereis diversicol. Arch. Entw. Mech., Bd. 25.

ROMANES, G. J. 1895. Jelly-fish, starfish, and sea-urchins, etc. International Scientific Series, vol. 49.

RUBIN, R. 1903. Versuche über die Beziehung des Nervensystems zur Regeneration bei Amphibien. Arch. Entw. Mech., Bd. 16.

STEELE, M. I. 1907. Regeneration in the compound eyes of crustacea. Jour. Exper. Zool., vol. 5.

STOCKARD, C. R. 1908. Studies on tissue growth. III. An experimental study of the rate of regeneration of *Cassiopea xamachana*. Carnegie Inst. Wash. Pub. No. 103.

TSCHERMAK, A. VON. 1910. Ueber tonische und tropische Innervation. Folia Neuro-Biologica, Bd. III.

WALTER, F. K. 1911. Welche Bedetung hat das Nervensystem für die Regeneration der Tritonextremitäten? Arch. Entw. Mech., Bd. 33.

WINTREBERT, P. 1903. Sur la Régénération chez les Amphibiens des membres postérieurs et de la queue, en l'absence du système nerveux. C. R. Acad. Sci., T. 137.

———. 1904. Sur la Régénération des membres postérieurs chez l'axolotl adulte après ablation de la moelle lombo-sacré. Compt. Rendus. Soc. Biol., T. 56.

WOLFF, G. 1895. Entwicklungsphysiologische Studien. Arch. Entw. Mech., Bd. 1.

———. 1901. Entwicklungsphysiologische Studien. Arch. Entw. Mech., Bd. 12.

ZELENY, C. 1906. The regeneration of an antenna-like organ in place of the vestigial eye of the blind crayfish. Science, n. s., vol. 23.

———. 1907. The effect of the degree of injury, successive injury, and functional activity upon regeneration in the scyphomedusan *Cassiopea xamachana*. Jour. Exper. Zool., vol. 5.

VIII.

THE CHEMISTRY OF LIGHT-PRODUCTION IN LUMINOUS ORGANISMS.

BY E. NEWTON HARVEY,
Assistant Professor of Physiology, Princeton University.

One text-figure.

CONTENTS.

PAGE.

Introduction........................ 173

Studies on the ostracod crustacean *Cypridina hilgendorfii:*

General character of light and of luminous gland.................... 174
Nature of the light-secretion.......... 175
Porcelain filtration.................. 175
Lack of oxygen..................... 176
Desiccation and ether extraction...... 176
Chemical tests on the secretion....... 176
Luciferin and luciferase.............. 176
Photophelein and photogenin......... 178
Distribution of photophelein and photogenin in organisms................ 179
Dialysis........................... 183
Adsorption........................ 183
Temperature....................... 183
Stability.......................... 184
Preservatives and anesthetics......... 184
Protein precipitants................. 186
Acids and alkalies.................. 186
Potassium cyanide.................. 188
Saturation with sugar, $(NH_4)_2SO_4$, and NaCl.......................... 188
Concentration of photogenin and photophelein in *Cypridina*.............. 188
Is photogenin used up in light-production with photophelein?............ 189
Summary of results from *Cypridina*.... 191

Studies on fireflies:

Desiccation and extraction with fat solvents......................... 193
Extraction with aqueous solvents and effect of oxygen.................. 195
Distribution of photogenin and photophelein in the firefly and other organisms....................... 197
Concentration of photogenin and photophelein necessary to produce light.. 199
Properties of photogenin and photophelein.......................... 199
Summarising table.................. 200

PAGE.

Studies on a pennatulid, *Cavernularia haberi:*

General characters of the animal and of the photogenic material......... 202
Electrical stimulation................ 203
Lack of oxygen..................... 204
Reductase, oxidase, and catalase...... 204
Temperature....................... 205
Photogenin and photophelein......... 205
Chemical reactions.................. 207
Anesthetics........................ 207
Potassium cyanide.................. 208
Summary of results from *Cavernularia*.. 208

Studies on luminous bacteria:

Desiccation and extraction with fat solvents......................... 209
Effect of lack of oxygen.............. 212
Photogenin and photophelein......... 214
Temperature....................... 215
Oxidase and catalase................ 216
Luminous bacteria and osmotic pressure 216
Luminous bacteria and balanced solutions........................... 217
Effect of acids and alkalies........... 218
Effect of homologous alcohols (anesthetics).......................... 219
Effect of potassium cyanide.......... 220
Summary of results from luminous bacteria 220

Studies on a squid, ***Watasenia scintillans***:

General characteristics of light-production............................ 222
Photogenin and photophelein......... 223
Studies on a fish, ***Monocentris japonica***... 223

Studies on oxidative chemiluminescent reactions with substances of definite composition:

Historical......................... 224
The chemiluminescence of pyrogallol... 225
Significance for bioluminescence....... 232
Summary of study of chemiluminescent reactions 232
General conclusions.................. 233
Bibliography........................ 234

THE CHEMISTRY OF LIGHT-PRODUCTION IN LUMINOUS ORGANISMS.[1]

By E. Newton Harvey.

INTRODUCTION.

The literature on the subject of animal phosphorescence or bioluminescence or biophotogenesis is so voluminous that no attempt can be made in the present article to record a complete list. A fairly complete bibliography will be found in Mangold's article, "Die Production von Licht," in Winterstein's *Vergleichende Physiologie*. The names of Beijerinck, Dubois, Ehrenberg, Krukenberg, Mangold, McDermott, Molish, Panceri, Phipson, and Quatrefages are intimately connected with the chemical and physiological side of light-production. The work which has been accomplished up to the present time indicates that light-production by animals is to be classified among the chemiluminescent reactions which have been studied from the purely chemical side, especially by Radziszewski and Trautz. All of the evidence now indicates that oxygen is necessary for this reaction—at least the statement is true for the following investigated forms: the fireflies; luminous bacteria and fungi; *Noctiluca* (2); a pennatulid (*Cavernularia*); and an ostracod crustacean (*Cypridina*). It has been objected by some that if we place the organisms, say luminous bacteria, in an atmosphere devoid of oxygen and find that no light is produced, that merely means that certain functions of the cell are interfered with, *including* light-production, but does not indicate that oxygen is actually used up in the photogenic process. The objection will not hold, however, for extracts of luminous cells or the luminous secretion of *Cypridina*, which contains the light-producing substance in solution (so that it can not be removed by filtration through a porcelain or siliceous filter) will give no light if the oxygen be removed, and light returns when oxygen is readmitted.

We may therefore say that a definite substance is burned (oxidized), and in some forms it burns as granules and in others as a homogeneous and probably colloidal solution. This substance is stable and non-luminous when dried and is not a fat or any substance soluble in fat solvents. It has been called noctilucin by Phipson (6 and 7), photogen by Molish (8), luciferin by Dubois (9), and photogenin by myself. Phipson and Molish did not recognize that in many forms two substances are concerned in light-production, but Dubois (9) pointed out that in the elaterid beetles and in the mollusc *Pholas dactylus* such was the case and he showed that these substances could be separated by heat. I can confirm this statement for certain other forms (lampyrid beetles and *Cypridina*), but find that the substances in *Cypridina* have many characteristics different from those described by Dubois for *Pholas* and that the mechanism of light-production is quite different from that which Dubois (10) supposes.[1] A record of the experiments and a discussion of the chemistry of light-production in all its aspects will be found in the following pages. *Cypridina*, as the most favorable species, will be considered first and then the other forms which I have studied—the luminous beetles, the pennatulid *Cavernularia*, luminous bacteria, the squid *Watasenia scintillans*, and a fish (*Monocentris japonica*)—together with a consideration of certain chemiluminescent reactions which involve the taking up of oxygen.

---

[1] This paper is a compilation with additions and some changes of my work on luminous forms during the past three years. The original papers will be found grouped together in the bibliography (1), and are not specifically referred to in the text.

Most of the studies reported herein were made in Japan, a region unusually rich in luminous forms, through the kindness of Dr. Alfred G. Mayer and under the auspices of the Department of Marine Biology of the Carnegie Institution of Washington.

The success of my investigations was largely due to the interest and kindness of Professor C. Ishikawa, of the Zoology Department, Agricultural College, Imperial University of Tokyo, and to Professor I. Ijima, of the Imperial University, who extended to me the hospitality of the marine biological laboratory at Misaki. I am also indebted to Dean Kozai and Professor Aso, of the Agricultural College, for the use of many chemicals and apparatus. I extend my cordial thanks to all of these men.

STUDIES ON THE OSTRACOD CRUSTACEAN C. HILGENDORFII.

GENERAL CHARACTER OF LIGHT AND OF LUMINOUS GLANDS.

Cypridina hilgendorfii is a strongly negatively heliotropic ostracod best obtained at night by lowering into the water fish heads, on which the animals feed. It may be caught the year round, but is most abundant during August and September in Sagami Bay, Japan. Another non-luminous species (*Cypridina x.*) is often obtained from the fish heads together with *C. hilgendorfii*. It is positively heliotropic to lamplight. *C. hilgendorfii* is so strongly negatively heliotropic that it is not readily caught on moonlight nights and avoids a shore with many electric lights.

The light-giving material of *Cypridina* is a secretion formed in a special gland of yellow spindle-shaped cells, opening near the mouth.[2]

---

[1] All of Dubois's papers are listed in Ann. d. la Soc. Linn. de Lyon for 1913. A general account is given in "La Vie et la Lumière" Alcan, Paris.

[2] For a description of the anatomy of the organ see a paper by N. Yatsu in Journ. Morph., 1917, and Dahlgren (19).

In adult living cypridinas the gland-cells form a cylinder about 0.64 mm. by 0.24 mm., having a volume of about 0.0003 c.c. The secretion is readily formed upon agitation of the animal and is easily visible in a room in the daytime. To the dark-adapted eye the color is decidedly bluish and the light very intense. To a partially light-adapted eye the color is blue. In this respect the light differs markedly from the yellowish white (to the dark-adapted eye) light of luminous bacteria of *Cavernularia* or of the Japanese firefly, which is green to the partially dark-adapted eye. Lund (3) describes a *Cypridina squamosa* (?) and a *Cyclopina gracilis* from Montego Bay, Jamaica, which gave an "intensely luminous greenish yellow secretion." Most observations, however, record the light to be decidedly bluish. There is no inhibition of the light secretion in daylight, as has been described for other forms (4 and 5). When first caught, cypridinas give off the secretion readily, sometimes upon mere contact with the surface film of water, but if they are kept in the laboratory the secretion is not given off so readily. Removal from sea-water also inhibits the ejection of the secretion and it is necessary to squeeze the animal rather strongly before the secretion is given out. Electrical stimulation also calls forth the production of an abundant light-secretion.

NATURE OF THE LIGHT-SECRETION.

The gland-cells are almost completely filled with yellow material which can be observed to be composed of yellow globules 2 to 6 micra in diameter. These are extruded and dissolve to a colorless solution, absolutely free of visible granules, which gives the light. The globules are rather fluid in consistency, as the outline can sometimes be seen to pass through amœboid-like changes.

If we examine the natural secretion of *Cypridina* in sea-water at night, the light appears perfectly homogeneous. No points of light appear, such as might be due to granules, a condition wholly different from that in the juice of *Cavernularia*, which contains visible granules, and at night the light from these under the microscope gives the appearance of a starry sky.

If cypridinas be ground in a mortar and then examined at night, numerous very bright points of light appear, and these are greatly increased by the addition of fresh water to the preparation. The light undoubtedly comes from solution of the above-described yellow globules and when they have dissolved no more points of light, but a steady homogeneous glow, is to be observed. Electrical stimulation by strong interrupted induced shocks does not intensify the light from an extract of cypridinas, glowing faintly.

PORCELAIN FILTRATION.

Although no visible granules are found in the natural secretion of *Cypridina*, an ultramicroscopic investigation was not undertaken on account of lack of apparatus.

All of the light-producing substances (both photogenin and photophelein, p. 178) will easily pass a Pasteur-Chamberland or a Berkefeld filter tube, so that the particles present, if any, must be exceedingly small.

Complete proof of the truly soluble character of the light-producing substances is given by dialysis experiments recorded on page 183.

LACK OF OXYGEN.

Oxygen is necessary for light-production, as may be seen by placing the crushed animals in a hydrogen atmosphere, or by bubbling hydrogen through a glowing extract of the animals. The light never completely disappears, even after a long time, but remains dim, so that very little oxygen (as no special precautions were taken to remove the last traces of oxygen from the hydrogen, prepared in a Kipp generator) is sufficient to give light. On readmitting oxygen, however, a brilliant glow results. Unlike the firefly (p. 196), an extract of *Cypridina* can be kept in absence of oxygen for over ten days with no light-production, but light again instantly appears if oxygen is readmitted.

DESICCATION AND ETHER EXTRACTION.

Cypridinas may be dried over $CaCl_2$ and will give a brilliant light if crushed and moistened with sea-water. Dried crushed cypridinas may be extracted with six changes of ether during the course of two days without impairing in the least their power to produce light when again moistened. The luminous substance is therefore of a non-lipoid ether-insoluble nature, as might be expected from the fact that it is extruded from the animal as a clear water-soluble non-fluorescent secretion.

CHEMICAL TESTS ON THE SECRETION.

Despite the fact that the light from the natural secretion of *Cypridina* is very bright, a sample of the secretion, collected by shaking many cypridinas in a small volume of sea-water and filtering, responds to none of the common biochemical tests. It gives no precipitate with picric acid or on saturation with $(NH_4)_2\ SO_4$ or on boiling, even when made slightly acid. Fehling's reaction is negative, as also the biuret and xanthroproteic for proteins, the Molish reaction for carbohydrates, and the indophenol test (α-naphthol and para-phenylen-diamine) $+H_2O_2$ for oxidases.

I do not mean to infer from this that the luminous substance is neither protein, fat, or carbohydrate, but merely that the concentration giving a bright light is too small to respond to chemical tests (see p. 188).

LUCIFERIN AND LUCIFERASE.

The light from the natural secretion of *Cypridina* lasts for some time in sea-water and then disappears, and no amount of shaking will cause it to appear again. If we add to this natural secretion an extract of *Cypridina* heated to boiling, a brilliant light again results; or if we mix a water extract of *Cypridina* whose light has disappeared on standing with a similar extract whose light has been destroyed by boiling, light again results. As already mentioned, Dubois first demonstrated this for *Pyrophorus* and a year later for *Pholas dactylus*. In Dubois's phraseology we have mixed two substances—luciferin (in the boiled tube) and luciferase (in the tube allowed to stand)—necessary for light-production. According to Dubois (10), one of these, luciferase, is an oxidizing enzyme and is destroyed by heat; the other, luciferin, a substance not destroyed by heat, is capable of oxidation with light-production by means of luciferase. When the natural secretion of *Cypridina* is allowed to stand all the luciferin is oxidized and the luciferase is left; when a luminous extract of *Cypridina* is boiled the luciferase is destroyed before all the luciferin is oxidized. The two substances alone in solution are non-luminous.

At one time I believed Dubois's interpretation of this experiment to be correct, but results on *Cypridina* have led me to wholly different conclusions regarding the existence of luciferin and luciferase. Dubois's interpretation is indeed attractive. We know that the light-production is an oxidation; that two substances are concerned; that these substances give light in very small concentration comparable with enzyme activity (see p. 188); that one of them can use up a large amount of the other (see p. 189) and possesses certain properties (destruction by heat, phosphotungstic and tannic acid) characteristic of enzymes. Further, we actually know of oxidative reactions taking place with the production of light under the action of oxidizing enzymes from plants and animals (see p. 225).

It is quite possible that light-production in *Pholas dactylus* is of this nature, as it differs radically in very essential points from the mechanism in *Cypridina* and in the firefly. Thus, Dubois finds that *Pholas* luciferin (the substance not readily destroyed by heat and giving light with luciferase) can be oxidized with light-production by many oxidizing agents—blood, H_2O_2, $KMnO_4$, BaO_2, PbO_2, etc.—and that it occurs only in the luminous organs of *Pholas dactylus* (10). I find that *Cypridina* luciferin (a substance not readily destroyed by heat and giving light with luciferase) will not give light with the above-mentioned oxidizing agents and is found in many other non-luminous animals and in the non-luminous parts of *Cypridina hilgendorfii*.

Dubois finds that *Pholas* luciferase (a substance destroyed easily by heat and using up *Pholas* luciferin with light-production) occurs in many other non-luminous animals (10), whereas I find a body with the same properties only in the luminous organs of *Cypridina*. This substance, which we may temporarily call *Cypridina* luciferase, in concentrated solutions, will give light (as before mentioned) with extracts of many non-luminous animals. It will also give light if mixed with many pure substances, as chloroform, ether, benzol, thymol, saponin, oleic acid, atropin, NaCl, and others. Since most of the above substances could not possibly be oxidized by the luciferase, I conclude that they cause in some way the giving out of light in what Dubois terms luciferase. On this view luciferase is the source of the light and the luciferin (in the boiled extract of *Cypridina*) is something which causes the luciferase to emit light.

The substances causing the emission of light from a concentrated solution of luciferase are similar to those producing cytolysis of cells, and I have considered the possibility that the concentrated extract might contain fragments of the luminous gland-cells which cytolyze with light-production or possibly granules which dissolve with light-production, as described in many other forms, and as especially prominent in the juice of *Cavernularia*. I am, however, convinced that there are in the extract of cypridinas which will give light with unoxidizable substances no cell fragments present and no granules above those of submicroscopic colloidal dimensions, for the following reasons:

First, the light is always perfectly homogeneous and in marked contrast to the points of light of *Cavernularia* juice, where visible granules and cell fragments do occur.

Second, *Cypridina* extract (luciferase) will give light with thymol, butyl alcohol, or NaCl crystals after filtration through a Chamberland or a Berkefeld filter, which removes all cell fragments.

PHOTOPHELEIN AND PHOTOGENIN.

I conclude, therefore, that Dubois's luciferase, a thermolabile substance in luminous cells, is the light-producing body. It gives light in contact with many substances not necessarily oxidizable and an especially bright light with a thermostabile substance (Dubois's luciferin) found in high concentration throughout the body of *Cypridina hilgendorfii* and in small concentration in the blood or juices of other non-luminous invertebrates. I therefore propose the new names photogenin (light-producer, from φῶς, light, and γεννάω, produce) for luciferase and photophelein (light-assister, from φῶς, light, and ὠφελέω, assist), for luciferin.

As we shall see later (p. 189), the photophelein can not be regarded as an enzyme, because it is used up in the reaction and will not give light with an indefinite amount of photogenin. The photogenin corresponds much more to an enzyme, but it is also slowly used up. At present we can only speak of photophelein as a definite substance causing in some way the emission of light from photogenin.

Perhaps the comparison of the important facts concerning light-production in *Pholas* and *Cypridina* shown in table 1 will make clearer Dubois's and my own views, and bring out the contradictory properties of the corresponding substances in the two animals. A more complete table is given on page 200.

Whatever the exact interpretation of the facts may be, it is certain that two substances are concerned in light-production, and these may be separated, because they are destroyed at different temperatures. We may now inquire into each of their properties separately and return to a discussion of the mechanism of light-production, in considering the possible enzyme nature of photogenin.

TABLE 1.

	Pholas.		Cypridina.	
	Luciferin (oxidises with light-production by luciferase).	Luciferase (enzyme accelerating oxidation of luciferin).	Photophelein (substance assisting in production of light by photogenin).	Photogenin (auto-oxidisable in presence of photophelein).
Destruction by heat.....	−	+	−	+
Dialyzable..............	+	−	+	−
Stability................	+	−	−	+
Occurrence in non-luminous parts............	−	+	+	−
Oxidised with light-production by $KMnO_4$, H_2O_2, etc.............	+	−	−	−

DISTRIBUTION OF PHOTOPHELEIN AND PHOTOGENIN IN ORGANISMS.

Certain fundamental facts for the chemical theory of light-production appear when we study the distribution of photophelein and photogenin: (I) in the non-luminous parts of *Cypridina*; (II) in the non-luminous *Cypridina*; (III) in other non-luminous organisms; (IV) in luminous organisms.

By a careful, quick scissors-cut, the head end of *Cypridina* containing the luminous gland can be separated from the posterior half without any contamination of the latter with luminous secretion. If we now test the non-luminous half with dilute photophelein and photogenin, we find that it contains nothing which will give light with photophelein, but something which will give a bright light with photogenin. We must try the experiment immediately, because this substance disappears if the extract stands in presence of oxygen. In absence of oxygen, or if the extract is boiled immediately (but not too long a time), the substance does not completely disappear, even after an hour. There is, therefore, in the non-luminous parts, the substance photophelein, which disappears even in the absence of photogenin (from luminous gland), unless the solution be boiled or oxygen excluded. (Note the similarity to the disappearance of photophelein in the presence of photogenin, with this exception, that no light is produced.) The experiment seems to indicate a third substance destroyed by heat, in non-luminous parts, which oxidizes (since oxygen is necessary) the photophelein.

In the extract of the non-luminous species, there is also a similar substance (photophelein) which will give light with photogenin. Unlike the photophelein from the non-luminous part of *Cypridina hilgendorfii*, it occurs in very small concentration, so that we must use concentrated[1] photogenin and concentrated extract of *Cypridina x*.

TABLE 2.

Animal.	Order.	Character of light from juice.	
		Unboiled.	Boiled.
Sepia esculenta (blood)	Cephalopoda	Fair	Fair.
Dolabella sp. (blood)	Gasteropoda	Faint	Fair.
Laonome japonica (blood)	Annelida	Very faint	Very faint.
Panulirus japonica (blood)	Decapoda	Very faint	Faint.
Lepas anatifera (extract)	Cirripedia	Good	Bright.
Tetraclita porosa (extract)	Cirripedia	Faint	Fair.
Mitella mitella (extract)	Cirripedia	Faint	Fair.
Oxyrhynchidæ (moss crab) (extract)	Decapoda	Faint	Fair.
Palæmon sp. (extract)	Decapoda	Faint	Faint.
Chiton (extract)	Amphineura	Fair	Good.
Hormithoe imbricata (extract)	Annelida	Fair	Fair.
Cirratulus dasylophius (extract)	Annelida	Very faint	Very faint.
Marphysa iwamusi (extract)	Annelida	Faint	Faint.
Onchidium sp. (extract)	Gasteropoda	Faint	Faint.
Non-luminous parts firefly (extract)	Insecta	Negative	Fair.
Flat worm (sp.?) (extract)	Turbellaria	Faint	Faint.

The photophelein from *Cypridina x* is also destroyed or disappears if the extract stands 1½ hours in contact with oxygen, but not in the absence of oxygen. Boiling makes the extract more stable.

In some other non-luminous forms, widely different in relationship from *Cypridina*, there are substances which give light with concentrated photogenin and others which will not, whether the extract has been boiled or left unboiled. The extracts were boiled in order to destroy substances which in turn might quickly destroy photophelein.

Among the light-giving extracts may be mentioned those listed in table 2.

Of these forms, *Lepas* and *Chiton* gave the best light and of these two only *Lepas* gave light with dilute *Cypridina* photogenin. Too much stress must not be laid upon comparative results, because much depends upon the concentration and it is not easy to obtain extracts of comparative concentration.

---

[1] By concentrated photogenin is meant an extract of 1 *Cypridina* in 0.5 to 1.0 c.c. water.

Unlike the photophelein from the luminous parts of *Cypridina* or from non-luminous cypridinas, that of *Lepas* extract or of *Dolabella* blood is perfectly stable and will give light with photogenin even after standing for a period of 24 hours.

Many extracts were found to give no light with concentrated photogenin. These included those shown in table 3, which were tried both boiled and unboiled. Again it is possible that with greater concentrations even these extracts would call forth a faint light.

The following fluids and dissolved protein substances also give no light when their solutions are mixed with photogenin: 50 per cent egg albumen; 50 per cent egg yolk; Na nucleate; Na nucleoproteinate; Witte's peptone; neutral meat peptone; dried ox-blood extract.

TABLE 3.

Animal.	Order.	Animal.	Order.
Modiolus sp.?	Lamellibranch.	Ligia exotica	Isopod.
Acmæa sp.?	Gasteropod.	Coccinella 7-punctata	Coleoptera.
Sistrum sp.?	Gasteropod.	Anomala rufscuprea	Coleoptera.
Sphærechinus pulcherrimus.	Echinoid.	Glysiphana jocunda	Coleoptera.
Wall crab (sp.?)	Decapod.		

It is certain, then, that there is photophelein or something similar to it in the blood or extracts of many invertebrates, but not necessarily in solutions of protein substances such as egg albumen, peptone, etc. In saliva there is something giving a very faint light and something in urine giving a fairly bright light with photogenin. Certain fluids tried were sufficiently acid to prevent the appearance of light and some fortuitous characteristic, such as acidity, may explain why extracts of some invertebrates give no light with photogenin.

It is hardly worth inquiring into the nature of the substances in each particular extract which may for convenience be collectively spoken of as photophelein, since I have found a great many simple bodies which, mixed with concentrated photogenin in powder or crystal form, give rise to a bright light. With more dilute solutions (one *Cypridina* to 10 c.c.) no light appears. These substances include sodium glycocholate, sodium taurocholate, thymol, chloroform, hydrochinon, NaCl, butyl alcohol, saponin, oleic acid, ether, benzol, atropin, pilocarpin, and ortol. Chloral hydrate, pyrocatechin, $FeSO_4$, $MgSO_4$, $(NH_4)_2SO_4$, esculin, dextrine, and $K_4Fe(CN)_6$ gave a fainter light, while saccharose gave none.

It is difficult to see in just what way all these diverse substances act. One is inclined to compare the production of light by chloroform or saponin to a process of cytolysis or to a stimulus reaction like artificial parthenogenesis. The photophelein of *Cypridina* or other extracts would then be comparable to the cytolytic substance in the blood of worms which causes development of sea-urchin eggs (11). The chloroform or saponin might be supposed to cause the solution of granules or globules (as can be observed in many cells) and the production of light to be connected with this solution process. But I have already given evidence (p. 178) to show that there are no granules unless ultramicroscopic ones in the light-secretion of *Cypridina*, so that we must look to another interpretation.

Of many animal extracts tried on both concentrated and dilute photophelein, none will give light. Neither have I been able to obtain light with any chemicals or the oxidizing substances—neutral H_2O_2, BaO_2, ox blood alone or with neutral H_2O_2 added, neutral potato juice alone or with neutral H_2O_2, $KMnO_4$, $K_2Cr_2O_7$, $FeCl_3$, or $K_4Fe(CN)_6$. As already pointed out (p. 177), this result is the direct opposite of the case in *Pholas dactylus* as described by Dubois.

There is, then, in the non-luminous parts of *Cypridina hilgendorfii* a large amount of photophelein, but only a small amount even in a closely related non-luminous form. There appear to be only small amounts in other luminous forms not closely related to *Cypridina*. For instance, *Cypridina* photogenin will give a faint light with firefly photophelein, but it is not nearly so bright as with *Cypridina* photophelein nor as bright as that given by non-luminous *Lepas* extract. Unfortunately, no closely related luminous crustacean was available, so that the degree of specificity of photogenin and photophelein could not be determined. At least we may say that *Cypridina* photogenin and photophelein are specific to a certain extent, but not in the strict sense of the word.

The following tabulation gives the luminous animals which have been tried with *Cypridina* in concentrated solution and the character of the light resulting. None gives light in more dilute solution.

Cypridina photophelein × firefly photogenin	Faint.
Firefly photophelein × *Cypridina* photogenin	Faint.
Firefly photophelein × firefly photogenin	Bright.
Cypridina photophelein[1] × *Cavernularia* photogenin	Fair.
Cavernularia photophelein × *Cypridina* photogenin	Negative.
Cavernularia photophelein × *Cavernularia* photogenin	Negative.
Cypridina photophelein × *Noctiluca* photogenin[2]	Faint.
Noctiluca photophelein[2] × *Cypridina* photogenin	Faint.
Noctiluca photophelein[2] × *Noctiluca* photogenin	Negative.

Owing to lack of material, the luminous fish *Monocentris japonica* could be tested only in more dilute solutions, and with negative results, viz:

Cypridina photophelein × *Monocentris* photogenin	Negative.
Monocentris photophelein × *Cypridina* photogenin	Negative.
Monocentris photophelein × *Monocentris* photogenin	Negative.

---

[1] An extract prepared with sea-water, as the dark juice of *Cavernularia* gives a brilliant light with fresh water.

[2] All the *Noctiluca* extracts were made with n/1000 NH_4OH to neutralize the acid of the *Noctiluca* vacuoles.

DIALYSIS.

Photophelein will dialyze through heavy parchment and collodion fairly readily, in the case of collodion sometimes appearing in the dialysate in the course of 2 hours.

Photogenin dialyzes with difficulty and with some collodion tubes not after a period of 36 hours. In others a very slight amount will pass in that time and in one experiment with heavy parchment paper a very slight dialysis occurred in 12 hours, but usually there was none. The collodion tubes and the paper did not leak in any of the experiments recorded.

ADSORPTION.

Both photophelein and photogenin are removed from solution by washed boneblack and washed freshly precipitated $Fe(OH)_3$. To serve as control the last washings from boneblack and $Fe(OH)_3$ were added to photogenin and photophelein and tested with photophelein and photogenin, respectively, to make sure that the adsorption was not apparent and due to destruction by foreign substances from the adsorbing media.

TEMPERATURE.

Lund (3) finds that in *Cypridina squamosa* (?) and *Cyclopina gracilis* the light from the luminous secretion disappears at 50° and if heated above 50° (as high as 70° but not above) it will again reappear at 50°. The time factor is always involved in determining the critical points in the effects of temperature, and I find that the concentration of the light-producing substances is also involved. Therefore the exact temperature of destruction of photephelein and photogenin depends upon their concentration and the time of heating. I find that cypridinas dried over $CaCl_2$, ground, and the powder suspended in sea-water give a beautiful light which disappears when heated to 56°, but returns on cooling. If heated to 65° and cooled, the light also returns, but does not return if heated to 70° and then cooled. A very concentrated mixture of photogenin and photophelein may be heated above 70° and whole cypridinas heated to boiling will occasionally give a faint light when cooled. The light from the normal secretion of *Cypridina* disappears at 52° to 54°, and returns on cooling, so that we may regard this as the inhibition temperature and something above 70°, depending on the concentration, as the destruction temperature. These results are in good agreement with those of Lund.

The time necessary to destroy photophelein at 100° depends also upon its concentration. In dilute solution (1 *Cypridina* to 25 or 50 c.c. water) boiling for 1 minute is sufficient, but in concentrated solution (1 *Cypridina* to 1 c.c. water) 5 minutes' boiling is necessary.

In *Pholas dactylus* (5) photophelein (luciferin) is destroyed above 70°, whereas in the firefly (both *Photuris* and *Luciola*) it may be boiled for 10 minutes without destruction.

The luminous material of *Cypridina*, like that of most luminous organisms, is unaffected by cold and will glow brilliantly at 0° C.

STABILITY.

Cypridina photogenin is much more stabile than photophelein. The time after preparation that one can obtain light from these substances depends largely upon their concentration, as also upon the temperature. One sample of concentrated (1 *Cypridina* to 2 c.c.) photogenin gave light with fresh photophelein for 7 days at 20°, despite the fact that decomposition had taken place and the liquid smelled foul. Dilute photogenin (1 *Cypridina* to 50 c.c.) may lose its power in 1 day; very dilute photogenin (1 *Cypridina* to 12,500 c.c.) in less than 2 hours.

Cypridina photophelein also disappears from solution spontaneously, and the more dilute the solution the more quickly does it lose its power to phosphoresce. With one *Cypridina* to 25 c.c. the power disappears in 3 to 4 hours, while with 1 *Cypridina* to 1 c.c. it may last for 5 days at 26° to 28° C.

The decomposition occurs whether the solution be neutral, acid (n/8000 HCl), or alkaline (n/4000 NaOH), in pure-water or sea-water, in the light and also in amber bottles, and occurs even if the photophelein be thoroughly boiled to destroy all traces of photogenin which might slowly use up the photophelein without light-production (see p. 189).

The photophelein is apparently oxidized, as the destruction is not so rapid in absence of oxygen.

TABLE 4.—*Effect of preservatives on photophelein.*

Saturation with—	Tested with photogenin after—			
	5 minutes.	1 hour.	1 day.	2 days.
Ether	Bright light	Negative	Negative	Negative.
Benzol	Bright light	Very, very faint	Negative	Negative.
Chloroform	Bright light	Very faint	Negative	Negative.
Thymol	Bright light	Faint	Negative	Negative.
Control	Bright light	Bright light	Faint[1]	Negative.

[1] Often photophelein will not last for one day.

PRESERVATIVES AND ANESTHETICS.

The addition of preservatives (anesthetics), as chloroform, ether, benzol, and thymol, is to hasten the spontaneous destruction of the photophelein and to preserve the photogenin.

Ether is especially destructive to the photophelein, whereas photogenin will give light after saturation with ether for 22 days. Tables 4 and 5 show the effect of saturation of solutions (1 *Cypridina* to 25 c.c.) of photophelein and photogenin with the four substances.

The harmlessness of the above anesthetics for *Cypridina* photogenin is unusual, as Dubois found a marked destructive action on *Pholas* photogenin (luciferase) and I have noted the same thing for the firefly.

As we have just seen, the addition of certain anesthetics does not rapidly destroy photophelein or photogenin. We can saturate a phosphorescent mixture of the two with ether and the light will still last for some time. If we add butyl alcohol to saturation the light disappears, and if the solution is now diluted with water or sea-water, the light reappears. The same phenomenon is observed if the photogenin be filtered through a Chamberland porcelain filter to remove all traces of cells or cell fragments. Care was taken to make sure that the return of light was not due to fluid adherent to the sides of the test-tube and untouched by the butyl alcohol.

TABLE 5.—*Effect of preservatives on photogenin.*

Saturation with—	Tested with photophelein after—					
	1 day.	2 days.	7 days.	22 days.	50 days.	130 days.
Ether	Bright	Bright	Faint	Very faint.	Negative.	
Benzol	Bright	Bright	Bright	Negative.		
Chloroform	Bright	Bright	Bright	Bright	Bright	Negative.
Thymol	Bright	Bright	Faint	Faint	Negative.	
Control	Bright	Faint	Negative.			

A similar phenomenon is observed with ethyl alcohol and acetone. If we add in small amounts absolute ethyl alcohol to a glowing mixture of photogenin and photophelein, the light becomes very dim when 16 per cent alcohol has been added and disappears with 20 per cent alcohol. If now the mixture be diluted, the light returns. Acetone[1] behaves as alcohol. About 23 per cent is necessary for extinction of the light. Saturation with chloretone does not extinguish the light.

The effect of ethyl alcohol and acetone might be explained as the effect of precipitation, because of insolubility in the 20 per cent solution, but we can not so explain the extinction of the light by butyl alcohol and subsequent recovery on dilution, since butyl alcohol is only soluble to the extent of 8.3 parts in 100 parts of water. We are dealing with a highly interesting effect—one akin to anesthesia—a reversible inhibition, not of a cell or cell fragments, but of a solution. Filtration through porous porcelain shows that no cell fragments can be present. I can not here enter more fully into a discussion of this interesting phenomenon, whose bearing on anesthesia is obvious, except to point out that if we can anesthetize a solution we need not, as some recent theories have done, regard changes in the cell-membrane to be necessarily the ultimate cause of anesthesia.

It may be pointed out in passing that the production of light gives us the opportunity of observing the effect of, let us say, an anesthetic or temperature upon a process at any particular instant, rarely obtained in the study of enzyme action or even of cell action, except where some movement is affected. For instance, we can heat pepsin to 60° and then cool it and see if it will digest protein. If we find that it will digest protein, that gives us no information as to a reversible inhibition at 60°, an inhibition which might become non-reversible if we kept the pepsin at 60° long enough to test directly the power of digestion. We can, however, heat photogenin and photophelein to 60°, cool them, and find out immediately if they give light.

---

[1] The sample of acetone at hand was not particularly pure.

PROTEIN PRECIPITANTS.

The light disappears in a phosphorescent mixture of photogenin and photophelein when the following substances are added to the concentrations indicated: tannin—m/2500 to m/5000; phosphotungstic acid—m/2500 to m/5000; picric acid—m/500, assuming a saturated water solution to be 1.03 per cent or m/22.

Table 6.—*Effect of acids and alkalies on light-production.*

Concentration in mixture.	Character of light.
n/250 NaOH	Instantly disappears.
n/500 NaOH	Instantly very dim, lasting >5 minutes.
n/1000 NaOH	Instantly dim, lasting >5 minutes.
n/2000 NaOH	Instantly fair, lasting >5 minutes.
n/4000 NaOH	Instantly fair, lasting >5 minutes.
n/8000 NaOH	Instantly fair, lasting >5 minutes.
Control	Remains good >5 minutes.
n/2000 HCl	Instantly disappears.
n/4000 HCl	Instantly dim, lasting >5 minutes.
n/8000 HCl	Remains good >5 minutes.

A peculiar phenomenon observed with phosphotungstic and tannic acids but not with picric acid is that, at the lower limit of extinction, the light reappears after first disappearing and more acid must be added in order to extinguish it again. Care was taken to make sure that the phenomenon was not due to more perfect adaptation of the eyes to the dark. When the light is extinguished by dilute picric acid it will reappear again on dilution with water.

ACIDS AND ALKALIES.

The effect of acids (HCl) and alkalies (NaOH) can be seen by adding 1 c.c. of the acid (or alkali) of a definite concentration to 1 c.c. of a glowing mixture of photogenin and photophelein. The concentration of the acid or alkali in the mixture will thus be one-half of the concentration added. The results are given in table 6.

The effect of acid (HCl) and alkali (NaOH) was also studied in the following way: 1 c.c. fairly dilute (1 *Cypridina* to 10 c.c.) photophelein was mixed with 1 c.c. n/500 HCl, allowed to stand 1 hour and then neutralized by adding an equal volume of n/1000 NaOH and tested for light by adding photogenin. The photophelein was therefore in contact with n/1000 HCl for 1 hour. In this way the effect of acid on photogenin and photophelein can be separated. The results are given in table 7.

TABLE 7.—*Effect of acid and alkali on photophelein and photogenin.*

Concentration of acid and alkali.	Effect on photophelein neutralised after 1 hour and photogenin added.	Effect on photogenin neutralised after 1 hour and photophelein added.
Concentration of HCl:		
n/1000	Negative	Negative.
n/2000	Very faint	Negative.
n/4000	Bright	Very faint.
n/8000	Bright	Bright.
Control in water	Bright	Bright.
Concentration of NaOH:		
n/31	Faint	Negative.
n/62	Faint	Negative.
n/125	Faint	Negative.
n/250	Faint	Faint.
n/500	Faint	Fair.
n/1000	Faint	Fair.
Control in water	Good, faint	Fair.

Even very small concentrations of NaOH added to a glowing mixture of photogenin and photophelein will decrease greatly the amount of light (table 6). Thus if we add 1 c.c. n/500 NaOH to 1 c.c. of a brightly glowing mixture, making the concentration of NaOH n/1000, the light instantly becomes very dim and remains so for a considerable time. If NaOH be added to a concentration of n/250, the light disappears completely; if we now neutralize the NaOH with n/250 HCl, the light returns, faint.

Still smaller concentrations of HCl prevent light-production. Thus (table 6) n/2000 HCl extinguishes the light from a luminous mixture, but on neutralization with n/2000 NaOH, the light returns and is fairly bright. The effect of NaOH and HCl is therefore to inhibit light-production, and not to immediately destroy the photogenin and photophelein (tables 6 and 7). The reversibility is not apparent if the acid or alkali are only slightly stronger than the values given. (Note also from table 7 that the photogenin is more readily affected by HCl and especially by NaOH than the photophelein.)

In the case of acid and alkali, as with so many other substances on the light-giving material, the effect is to an extent reversible if the substance is removed. The concentration of HCl which inhibits light-production is the same as that affecting cells, although the concentration of NaOH necessary to inhibit light-production is somewhat greater than that necessary to affect cells (12 and 13).

POTASSIUM CYANIDE.

The effect of KCN is of especial interest because of its power of inhibiting cell oxidations. It was tested by mixing an equal volume of photogenin (1 *Cypridina* to 25 c.c.) with the KCN solution, and testing with an equal volume of photophelein (1 *Cypridina* to 12 c.c.) after 10 minutes and after 1 hour. Table 8 gives the results.

TABLE 8.—*Effect of KCN on light-production.*

Concentration of KCN in photogenin solution.	Light with photophelein after 10 minutes.	Light with photophelein after 1 hour.
n/20	Fair.	
n/30	Fair.	Fair.
n/125	Fair.	Fair.
n/250	Bright.	Bright.

In *Cypridina*, as in all other luminous animals which have been tried (*Cavernularia*, *Noctiluca* (2), firefly, luminous bacteria), KCN is practically without influence on light-production. *Cavernularia* juice, for instance, will light for over 90 minutes in n/40 KCN. On the other hand, n/1280 KCN is sufficient to completely inhibit the oxyluminescence of pyrogallol by the vegetable oxidases (see p. 230).

SATURATION WITH SUGAR, $(NH_4)_2SO_4$, AND NaCl.

Saturation of a luminous mixture of photogenin and photophelein with sugar or NaCl causes the light to disappear and it reappears on dilution of the mixture with water.

Since the $(NH_4)_2SO_4$ used was acid, a small amount of this salt caused the light to disappear and it did not reappear on dilution of the mixture with water.

Probably these phenomena are connected with the salting-out of the luminous substances, although no signs of a precipitate are visible when the natural secretion of *Cypridina* is saturated with NaCl or $(NH_4)_2SO_4$. As already pointed out, this result is no doubt due to the small concentrations of the substances present.

CONCENTRATION OF PHOTOGENIN AND PHOTOPHELEIN IN CYPRIDINA.

In the normal secretion of *Cypridina* there is more photogenin than photophelein, as may be seen by adding fresh photophelein to the normal secretion after the light has disappeared on standing. The light again appears. The photophelein had been completely used up. This may be shown in another way by allowing a concentrated mixture of photogenin and photophelein to stand until the light disappears, and then boiling one-half of the mixture. Upon mixing the two halves no light results, as all the photophelein had been used up before the photogenin was destroyed in the tube boiled.

The concentrations of the two substances which will give visible light when mixed are very small. In one experiment performed 15 minutes[1] after the photophelein was obtained, 1 *Cypridina* in 25,600 c.c. of water gave a just-visible light when mixed with an equal volume of more concentrated photogenin. The photogenin from 1 *Cypridina* in 25,600 c.c. of water will also just give visible light when mixed with an equal volume of more concentrated photophelein.

We can see how small an amount of photogenin will give light when we consider that the animal is about 3.5 by 2.5 by 1.5 mm. and that the luminous gland is more or less of a cylinder 0.64 mm. high by 0.24 mm. diameter, with a volume of about 0.03 mm. or 0.00003 cm. and that 1 luminous gland in 25,600 c.c. water will give visible light when mixed with an equal volume of photophelein, or in a concentration of 51,200 c.c. Hence 1 part of gland in about 1,700,000,000 parts of water will give visible light upon the addition of photophelein. Even this is a low estimate, as we do not know what the concentration of the photogenin is in the gland itself.

When we compare the amount of photogenin which can be detected by light-production with the amount of substances detectable by chemical means, we see how extraordinarily sensitive the light-reaction is. AgCl is soluble in water to the extent of 1 part in 625,000 parts water at 20° (14), so that more chloride than this must be present to be detected by $AgNO_3$, a very delicate reaction. $KMnO_4$ is roughly just distinguishable by its color in a 14 mm. test-tube in a concentration of 1 to 1,250,000.

Biological reactions are, generally speaking, more delicate than inorganic reactions. Zinc in traces has a favorable influence on the growth of molds and 1 to 25,000,000 will increase the weight of a crop of *Aspergillus* by 50 per cent (15). Copper will kill *Spirogyra* in 1 minute when present in 1 to 77,000,000 concentration (15). According to Kastle (16), blood in 1 to 80,000,000 can be recognized by the phenolphthalein reaction. Since this reaction is due to hemoglobin, of which blood contains about 10 per cent, the hemoglobin can be recognized in 1 to 800,000,000 concentration. These figures give an idea of the delicacy of the photogenin-photophelein reaction as compared with others. It should be borne in mind that pyrogallol in 1 to 254,000 will give visible light with potato-juice and H_2O_2 (see p. 225).

IS PHOTOGENIN USED UP IN LIGHT-PRODUCTION WITH PHOTOPHELEIN?

The exceedingly small concentration of light substances which give visible light suggests that one or both are of enzyme nature, as Dubois supposes. There are two ways of testing this question. One is to determine whether a small concentration of photogenin can use up a large concentration of photophelein, providing a sufficiently long time is allowed. We can not use this method, because photophelein decomposes spontaneously.

---

[1] The experiment was performed as quickly as possible, because the photophelein disappears on standing.

Another way is to determine if a small amount of photogenin can decompose successively added amounts of photophelein without itself undergoing diminution. This method is not unequivocal, since many true enzymes are paralyzed or destroyed by the decomposition products of the reaction which they accelerate.

It was found that if we add to 1 c.c. of a weak (1 *Cypridina* to 50 c.c.) solution of photogenin, successive 1 c.c. additions of a concentrated (1 *Cypridina* to 2 c.c.) solution of photophelein as soon as the light from the preceding addition has disappeared, after four 1 c.c. additions no more light will appear. The photogenin is therefore used up and can not oxidize additional photophelein, although there is plenty of photophelein present, as may be shown by adding fresh photogenin, when a good light appears. With each successive addition of concentrated photophelein, the light, which at first is very bright and lasts about an hour, becomes less brilliant and lasts a shorter time. This is not due to mere dilution of the dilute photogenin, as we can dilute the dilute photogenin to the same volume with water and then upon the addition of photophelein a good light results. We can conclude only that, although photogenin can use up a large amount of photophelein, it is itself changed in some way in the reaction and disappears. We can not say how much photophelein will combine with a definite quantity of photogenin, because we do not know the absolute amount of these substances in a single *Cypridina*. In the above experiment we added a concentration of photophelein from one *Cypridina* 100 times (*i. e.*, four additions each 25 times more concentrated) that of the photogenin from one *Cypridina*.

Although the evidence goes to show that the photogenin is used up, it is not nearly so rapidly used up as is the oxidase of potato in the production of light from pyrogallol (see p. 231). We must remember also that certain enzymes, as zymase (thermolabile and non-dialyzable), are only active in presence of a coenzyme which is not destroyed by heat and is easily dialyzable. In fact, the photogenin-photophelein system resembles to a very remarkable degree the zymase-cozymase system. There is the same quantitative relation between zymase and cozymase as between photogenin and photophelein. If zymase is present in excess, the coenzyme is all used up; if cozymase is in excess then the zymase is used up (17). Nevertheless, however much photogenin resembles cozymase, I have for the present deemed it best to avoid the termination *ase*. In absence of more definite knowledge we may provisionally regard photogenin as a substance auto-oxidizable only in presence of photophelein.

If we try the reverse experiment, that of adding 1 c.c. of concentrated (1 *Cypridina* to 2 c.c.) photogenin to 1 c.c. of dilute (1 *Cypridina* to 50 c.c.) photophelein, a bright light appears lasting 10 to 15 seconds and no more light upon adding additional concentrated photogenin. As might be expected, the small amount of photophelein is very rapidly used up by the large amount of photogenin.

The quantitative relations between concentration of photophelein and photogenin, duration of light, and brightness of light will be considered in a subsequent paper.

SUMMARY OF RESULTS FROM CYPRIDINA.

1. The luminous secretion of *Cypridina* comes from several spindle-shaped yellow gland-cells on the upper lip and is extruded to the sea-water as a perfectly clear granule-free non-fluorescent secretion. The light is homogeneous and bluish white in color.

2. If the luminous secretion stands, the light disappears, and if we now add an extract of cypridinas heated to boiling, the light again returns—*i. e.*, *Cypridina* gives a luciferin-luciferase reaction similar to *Pholas dactylus*, as described by Dubois.

3. Contrary to Dubois's theory, the luciferase is the source of the light, and not an enzyme causing light-production by oxidation of luciferin, because we can obtain light from luciferase by substances incapable of oxidation (NaCl, chloroform, ether, etc.). The new names of photogenin or light-producer for luciferase and photophelein or light-assister for luciferin have therefore been proposed.

4. Oxygen is necessary for light-production.

5. Both photogenin and photophelein will pass a Pasteur-Chamberland or Berkefeld filter easily.

6. Photophelein dialyzes readily through heavy parchment or collodion, photogenin with great difficulty or not at all, even after a period of 36 hours.

7. Both photogenin and photophelein are adsorbed by boneblack and $Fe(OH)_3$.

8. The light-producing substances may be dried and thoroughly extracted with ether without impairing their light-giving power.

9. Chemical tests on the natural light-secretion give negative results, since a very small amount of light-substance gives a bright light and at least 1 part of photogenin or photophelein in 1,700,000,000 parts water will give a visible light.

10. Photophelein occurs throughout the body of *Cypridina*, photogenin only in the luminous organ. Photophelein from non-luminous parts disappears (apart from photogenin) on standing, but not so readily if the extract has been boiled or in the absence of oxygen. In the firefly and some non-luminous beetles the photophelein disappears so quickly that it is best to make the extract with boiling water. In

a non-luminous species of *Cypridina* a photophelein occurs in small quantity with properties similar to that in *Cypridina hilgendorfii.*

11. Photophelein occurs in extracts (both boiled and unboiled) of many non-luminous organisms, in greatest quantity in *Lepas anatifera.* The photophelein of *Lepas anatifera* does not disappear on standing.

12. Pure protein solutions (peptone, Na nucleoproteinate, albumen, etc.) or dried mammalian blood do not contain photophelein, but urine contains a similar body.

13. While photogenin will give light with many substances known and unknown, photophelein will give light only with the photogenin of luminous organs.

14. *Cypridina* photogenin will give no brighter light with extracts (photophelein) of other luminous forms (*Luciola, Cavernularia, Noctiluca*) than with extracts of non-luminous forms, and *Luciola* photogenin will not give so bright a light with *Cypridina* photophelein as with boiled extracts of non-luminous insects. We must conclude that the two substances are not specific for luminous forms, although there is a certain amount of specificity, for photogenin gives the best light with photophelein from the same species.

15. Bright light can be formed by *Cypridina* at 0° C. Photogenin is destroyed above 70°, the temperature and time depending on the concentration; photophelein only after several minutes' boiling, the time depending also on the concentration. The natural luminous secretion ceases to light at 52° to 54°, but the light returns on cooling.

16. Photophelein is relatively unstabile and disappears on standing, the time depending on the concentration. Photogenin is much more stabile, but also disappears slowly.

17. The spontaneous decomposition of photophelein is retarded (perhaps prevented) by lack of oxygen, but hastened by addition of preservatives (chloroform, ether, benzol, thymol). Photogenin can be kept longer by addition of preservatives. One sample preserved with chloroform retained its power to give light with photophelein for over 56 days at room temperature.

18. Saturation with ether, chloroform, benzol, thymol, or chloretone does not affect the light from a mixture of photogenin and photophelein. Saturation with butyl alcohol or 20 per cent ethyl aclohol or 16 per cent acetone extinguishes the light, and if the mixture is diluted with water the light reappears. This phenomenon of anesthesia of a solution is given by photogenin filtered through porous porcelain plus photophelein and difficultly soluble butyl alcohol, so that it can not be due to the presence of cell fragments or to insolubility in the 20 per cent ethyl alcohol.

19. Picric acid, tannic acid, and phosphotungstic acid extinguish the light in very weak concentrations, and the light returns in the case of picric acid if the solution is diluted with water. In the case of

tannic and phosphotungstic acids, the light returns after first disappearing, even without diluting.

20. HCl between n/2000 and n/4000 and NaOH between n/250 and n/500 extinguish the light, and the effect is reversible upon neutralization. It can be shown that both HCl and NaOH affect the photogenin more readily than the photophelein and that the effect of NaOH especially is more readily reversible.

21. KCN does not inhibit light-production even in strong concentration.

22. Saturation with $(NH_4)_2SO_4$, NaCl, or cane-sugar extinguishes the light most readily in the order named, and the effect is reversible except in case of $(NH_4)_2SO_4$ (due to acidity).

23. In the natural secretion of *Cypridina* or in the whole animal there is always enough photogenin to completely use up the photophelein. The photogenin from one animal will use up a large additional amount (at least 100 times the concentration in one animal) of photophelein, but not an indefinite amount, so that photogenin is not a true enzyme in the strict sense of the word, unless it be an enzyme poisoned by its own reaction products. The photogenin-photophelein system resembles the zymase-cozymase system to a remarkable degree, but it is best for the present to regard photogenin not as an enzyme but only as a substance auto-oxidizable only in presence of photophelein.

STUDIES ON FIREFLIES.

DESICCATION AND EXTRACTION WITH FAT SOLVENTS.

The experiments recorded herein have been made during a period of two years on several different species of firefly—the American *Photuris pennsylvanica* and *Photinus pyralis*, the Japanese *Luciola vitticollis* and *L. parva*, and the West Indian "cucullo," *Pyrophorus noctilucus* and *P. havaniensis*. All the species are essentially similar in behavior, and in general the statements apply to all.

Contrary to the condition in *Cypridina*, the photogenic substance is burnt within the cell which forms it and is found there in the form of granules scattered through the cytoplasm. The histological structure of the luminous tissue has been well described by many observers [Townsend (18), Lund (3), Dahlgren (19), Vogel (43), Bongardt (44), Geipel (45), Williams (46)] and need not be considered here.

The old observation that firefly luminous tissue can be dried and ground up and will phosphoresce when water containing oxygen is again added, gives a simple chemical method of investigating the nature of the photogenic material. The dried material may be extracted with water-free solvents (since the photogen does not oxidize in absence of water) and extracted material as well as the residue from evaporation of the filtrate may be tested for phosphorescence by adding water. The results indicate that a large number of fat solvents will extract nothing from the dried tissue and leave the photogenic material unharmed. Indeed, the material may be extracted with boiling ether for 24 hours without impairing its power to phosphoresce. Boiling alcohol does destroy the power to phosphoresce, and the nature of its action is discussed below. These results, as well as the previous experiments of McDermott (20 and 21) and Dubois (22), using fresh watery material, show conclusively that the photogenic substance is not a fat or fat-like body of any kind. The results are given in table 9, which also gives the time of extraction and the temperature.

TABLE 9.

Substance.	Temperature (in degrees).	Time (in hours).	Extracted material.	Extract evaporated in vacuo.
Ether (cold)	20	72	+	−
Ether (hot)	35	24	+	−
Chloroform (cold)	20	72	+	−
Chloroform (hot)	61	8	+	−
Ethyl alcohol (cold)	20	24	+	−
Ethyl alcohol (hot)	78.4	24	−	−
Ethyl alcohol and ether (equal parts) boiling	44	10	+	−
Carbon tetrachloride	20	48	+	−
Carbon disulphide	20	48	−	−
Acetone	20	48	+	−
Toluol	20	48	+	−
Amyl alcohol	20	48	Very faint[1]	−
Ethyl butyrate	20	48	Very faint[1]	−

[1] The material was washed with ether to remove the amyl alcohol and ethyl butyrate.

A plus sign indicates phosphorescence when water is added and a minus sign indicates no phosphorescence. Both the original extracted material and the residue of the filtered extract evaporated to dryness were examined. The results indicate not only that the photogenic substance is not a fat, but also not a lecithin. I am aware that the lecithins are difficult to extract *in toto* from the cell, but this can be accomplished by a mixture of hot ether and alcohol, and yet a mixture of hot ether and alcohol will extract nothing which will phosphoresce from the firefly powder. We may safely say that the photogen is not a lecithin.

Of all the solvents tried, only hot alcohol and cold amyl alcohol and ethyl butyrate gave results that would indicate a possible solution of the photogenic substance; and yet there is nothing in the evaporated filtrate that will phosphoresce when water or a neutralized 3 per cent solution of H_2O_2 is added. Thinking that a second substance might be necessary and that this had not been extracted by the fat solvents, although the photogen had, the filtrate was also tested by adding a water extract of firefly organs, fresh or preserved with toluol or chloroform, and also by potato-juice which contains considerable quantities of oxidizing enzymes. In no case was phosphorescence observed. The boiling ethyl alcohol,[1] cold amyl alcohol, and ethyl butyrate must, therefore, break up the photogen. It is the alcohol itself and not the temperature (78.4°) of boiling alcohol which is responsible for the destruction of the photogen, as the dried powder will withstand this temperature for 24 hours without any appreciable diminution in its power to phosphoresce. McDermott finds that liquid sulphur dioxide and liquid ammonia also destroy the photogenic power (20).

EXTRACTION WITH AQUEOUS SOLVENTS AND EFFECT OF OXYGEN.

If the luminous tissues of the firefly are extracted with water a solution which still gives light on filtration through filter paper is obtained. The light lasts a certain time and then disappears.

If the dried powdered luminous tissues of the firefly are allowed to come in contact with oxygen-free water, no light is produced, but if we admit oxygen, in a few minutes we find the mixture becomes luminous. If we wait for an hour or more before admitting oxygen, no light is produced. The apparatus shown in figure 1 is convenient for demonstrating this.

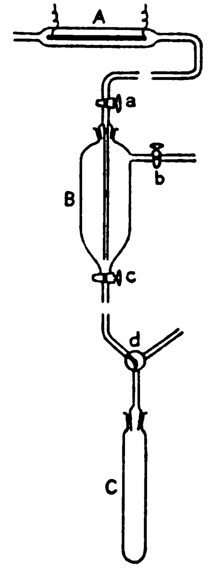


Fig. 1.—Apparatus for extraction of luminous tissue in absence of oxygen.

The material to be extracted is placed in the vessel *C* (fig. 1), provided with a ground-in stopper connected with a 120° stopcock. The water to be rendered free of oxygen is placed in *B* after passing hydrogen through stopcock *C* and closing it. *B* is connected through *A* with a hydrogen generator. The hydrogen is passed through potassium hydroxide to remove acid and then over a glowing platinum wire (in *A*) to remove the last traces of oxygen, a much better method than passing the gas through alkaline pyrogallol. By alternately exhausting *B* through *b*, connected to an air-pump, and refilling with hydrogen several times, the water can be quickly rendered free of oxygen. *C* is then connected to *B* through *c* and one of the arms of the 120° stopcock (*d*) whose other arm is connected with an air-pump. *C* and the arms of *d* are then exhausted. The 120° stopcock is then turned to connect *C* and *B* and *c* is opened, allowing the pressure of the hydrogen to drive the solvent on the material in *C*. The proper amount of fluid for extraction should be placed in *B*, so that the hydrogen may follow it through and fill the chamber *C*. Then *d* is closed, when *C* can be disconnected and shaken during extraction. To filter the extract it is only necessary to connect one of the arms of *d* with a desiccator fitted with a funnel and filter rack. When the desiccator is exhausted, *C* and the desiccator are connected, and the pressure of the hydrogen in *C* drives the extract onto the filter paper. The firefly photogen begins to phosphoresce when the atmospheric pressure reaches 5 to 6 mm., which means an oxygen pressure of 1 to 1.2 mm. Consequently it is necessary to use a good vacuum-pump and make connections air-tight. I found small-bore lead tubing sealed with Khotinsky cement the best for the purpose.

---

[1] The 99.8 per cent absolute alcohol was distilled over metallic calcium and collected in a receiver protected from the air by $CaCl_2$ in order to remove the last traces of water.

All of my experiments have been carried out in the dark and the material observed at critical stages (as when the oxygen-free water was added) to make sure that no light appeared, and always with negative results. But to make sure that no very slow leakage of oxygen into the filtering chamber occurred, I have carried out the extraction in a special tube provided with a capillary sealed off during the extraction. After extracting in this tube for 1½ hours and admitting oxygen, no phosphorescence appeared. Thinking that possibly the photogen dissolved in the extracting fluid did phosphoresce, but only so faintly as to be invisible because distributed through a relatively large volume of extract fluid, the unfiltered extract was evaporated *in vacuo* to a small volume. This can be very easily done by placing the rubber tube from the vacuum-pump over the capillary onto the special tube, exhausting, and then breaking the capillary through the walls of the rubber tube to connect with the air-pump. Even when concentrated the extract gave no light on adding oxygen.

We must conclude that the photogen is destroyed in distilled water, even without oxidation. The search for watery solvent for the photogen becomes, then, a search for a solvent in which the photogen is stable. The following solutions were tried in addition to distilled water. Extraction was allowed to proceed for from 1 to 1½ hours.

1. Ringer's solution (as representing fairly accurately the concentration and composition of the firefly's blood).
2. 0.125 m NaCl.
3. Sea-water (a mixture of chlorides and sulphates of Na, K, Ca, and Mg).
4. 5 per cent NaCl.
5. 0.05 m NaOH and 0.1 m NaOH. The dried, powdered firefly organs will phosphoresce strongly if sprinkled on the surface of 0.1 m NaOH.
6. 0.02 m HCl. Dried firefly powder will phosphoresce on 0.0125 m HCl and on 0.025 m HCl, but less brilliantly. Only one or two bright dots appear on 0.05 m HCl and no phosphorescence occurs on 0.1 m HCl. If neutralized within two minutes after contact with the acid, the light does not appear in the 0.1 m HCl treated material nor become brighter in the 0.05 m and 0.025 m treated material.

In each case, after extraction, oxygen was admitted and the solution shaken, yet in no case did light appear either in the undissolved residue or in the solution. The 0.02 m HCl extract was also neutralized, as it is well known that the acid prevents light-production. As McDermott (22) has simultaneously failed to extract a photogenic substance with oxygen-free aqueous solvents, we may safely conclude that something connected with light-production undergoes decomposition on standing. We shall see later what this substance is.

In 1885 Dubois (9) showed that the elaterid beetle *Pyrophorus noctilucus* contained the substances luciferin and luciferase which are described on page 176. I find that the lampyrid beetles *Photinus*, *Photuris*, and *Luciola* also contain similar substances, but their properties agree with the related bodies previously described in *Cypridina* rather than those described by Dubois for *Pholas* (10), and consequently I have likewise called them photogenin (=luciferase) and photophelein (=luciferin). For instance, firefly photophelein can not be oxidized with light-production by oxidizing agents and is found in many non-luminous forms, the opposite of the condition said to hold for *Pholas* luciferin (=photophelein).

Like *Cypridina* photogenin, firefly photogenin is prepared by allowing an aqueous extract of the luminous organ to stand until the light disappears, and the photophelein is prepared by extracting the firefly with boiling water. Light appears on mixing the two substances. As we shall see, firefly photophelein alone in solution is very stabile, but, together with unboiled extract of luminous or non-luminous parts of the firefly, it is very unstabile. Therefore the photophelein is probably the substance which underwent decomposition when dried powdered luminous tissue was allowed to stand in contact with oxygen-free water for one hour in the experiments described above.

DISTRIBUTION OF PHOTOGENIN AND PHOTOPHELEIN IN THE FIREFLY AND OTHER ORGANISMS.

While photogenin is found only in the luminous gland-cells of the firefly, photophelein is distributed throughout the body of the firefly. An extract of the non-luminous parts of the firefly will give light with photogenin, but only if tested immediately the extract is made. An extract 10 minutes old is found to contain no photophelein unless it has been previously boiled. After boiling the photophelein can be kept for many days without decomposition. There appears to be something in the extract of non-luminous parts of the firefly which causes the photophelein to disappear unless the former is destroyed by heat. That this is the case can be shown by adding unboiled extract of the non-luminous parts to photophelein, when the latter disappears from solution. Because of failure to boil the non-luminous firefly extract, I had for a long time overlooked the existence of photophelein in regions other than the luminous gland. Lack of oxygen retards but probably does not prevent the disappearance of photophelein from non-luminous parts. Note that just as photophelein disappears in contact with photogenin and oxygen with light-production, so it also disappears in contact with this substance in non-luminous parts, but without light-production. In this respect the firefly agrees perfectly with *Cypridina.*

Among non-luminous forms we find some whose extract will give light with photogenin, whether boiled or unboiled; others whose extract will give no light, whether boiled or unboiled. The first mentioned presumably contain photophelein not readily destroyed by standing, the second are similar to the firefly, and the third may contain no photophelein or some substance very quickly destructive to photophelein or an excess of acid or perhaps merely unstabile photophelein. The exact reasons have not yet been worked out. The following extracts were tried:

Luciola photogenin × caterpillar (sp?) blood	Bright.
Luciola photogenin × caterpillar (sp?) blood, boiled	Bright.
Luciola photogenin × beetle (*Coccinella 7-punctata*) extract	Negative.
Luciola photogenin × beetle (*Coccinella 7-punctata*) extract, boiled	Bright.
Luciola photogenin × beetle (*Glysiphana jucunda*) extract	Negative.
Luciola photogenin × beetle (*Glysiphana jucunda*) extract, boiled	Bright.
Luciola photogenin × beetle (*Anomala rufscuprea*) extract	Negative.
Luciola photogenin × beetle (*Anomala rufscuprea*) extract, boiled	Negative.
Luciola photogenin × grasshopper (*Pachytylus danicus*) extract	Very faint.
Luciola photogenin × grasshopper (*Pachytylus danicus*) extract, boiled	Faint.
Luciola photogenin × myriapod (*Scolopendra* sp?) extract	Faint.
Luciola photogenin × myriapod (*Scolopendra* sp?) extract, boiled	Faint.
Luciola photogenin × ox-blood extract (boiled or unboiled)	Negative.
Luciola photogenin × neutral potato-juice (boiled or unboiled)	Negative.

Note that the oxidases of blood or potato-juice will give no light with photogenin and that they also will give no light with photophelein, even if we add H_2O_2. As we shall see later, although the oxidases can oxidize pyrogallol with light-production, they have nothing to do with light-production by animals (see p. 232).

As we have already seen (p. 182), *Cypridina* photophelein, if concentrated, will give a very faint light with firefly photogenin, and *vice versa.* Firefly photogenin and luminous bacteria prepared in the proper way (p. 214) or *Watasenia scintillans* photophelein (p. 223) also give a faint light, but the converse experiment fails to produce light. *Cavernularia* photophelein gives no light with firefly photogenin, but the converse experiment (p. 205) does. At most the light produced in any of these cross-experiments between distantly related species is very faint and again shows that photogenin and photophelein from different luminous forms possess a certain degree of specificity. As was to be expected the different combination mixtures of these substances in various genera of the Lampyridæ all give light as well as the reciprocal combinations between Lampyridæ and Elateridæ (*Pyrophorus*). Indeed, it may be found that the photogenins from different forms exhibit differences in light-giving power, depending on relationship, similar to the differences in the hemoglobins, or similar to the specificity of the precipitin reactions of different animals.

Photophelein, on the other hand, will not give light with extracts of any non-luminous forms or with any chemical substances.

CONCENTRATION OF PHOTOGENIN AND PHOTOPHELEIN NECESSARY TO PRODUCE LIGHT.

The amount of photogenin in the firefly is greater than the amount of photophelein necessary to combine with it; otherwise we would not be able to obtain photogenin, because it would be completely used up in combination with photophelein.[1] In some luminous animals (*Noctiluca, Cavernularia, Watasenia*) I have utterly failed to demonstrate a photogenin-photophelein reaction under conditions which should be favorable and after many attempts to demonstrate it. The explanation of this result may lie in the presence of equivalent amounts of the two substances.

Both photogenin and photophelein are found in much smaller concentration in the firefly than in *Cypridina*. The photogenin from one firefly (*Luciola parva*), whose average size is 8.2 by 3.1 mm., with luminous organ in the male 2.2 by 1.5 mm. and in the female 2.3 by 0.5 mm., will give just visible light in 1.6 c.c. water, and the photophelein from 1 firefly in 3.2 c.c. water. The greater possible dilution of the photophelein is due no doubt to the fact that the photophelein comes from the whole body, while the photogenin is derived only from the luminous gland. When we compare this with the crustacean *Cypridina*, which will give visible light in 25,600 c.c. or one part of luminous gland in 1,700,000,000 parts of water, we see that the firefly, despite the brilliancy of its light, is not a very powerful light-producer.

PROPERTIES OF PHOTOGENIN AND PHOTOPHELEIN.

As to the chemical nature of photogenin and photophelein, nothing definite is known, except that it is not a fat or fat-like substance. The photophelein is much the more stable substance and can be preserved, until attacked by bacteria, or with chloroform for over 6 months. It dialyzes readily through collodion, is not adsorbed by lampblack, and is not readily affected by ether and benzol. Photogenin, on the other hand, disappears in less than 5 hours at 25° C., is quickly destroyed by ether, benzol, and chloroform, and will not dialyze readily if at all.

In this ready destruction of photogenin by the fat-solvent anesthetics, the firefly resembles *Pholas dactylus* and differs markedly from *Cypridina*. One sample of *Cypridina* photogenin was preserved under chloroform for over 54 days and still gave light with photophelein.

Luciola photogenin is destroyed at about 42°, while the photophelein is still active after 10 minutes' boiling. A bright light is produced on mixing the two at 0° C. Exact destruction temperatures are difficult to determine, however, and Lund (3) finds that in *Photuris pennsylvanica*, if the intact luminous tissues are studied, the light disappears

---

[1] I have not actually made the experiment to show that photogenin is used up in giving light with photophelein, but it seems highly probable that this is the case in the firefly, as it is in *Cypridina*.

TABLE 10.—*Comparative properties of photogenin and photophelein in Cypridina, firefly, and Pholas.*

Property.	Cypridina.		Firefly.		Pholas (Dubois, 10).	
	Photophelein.	Photogenin.	Photophelein.	Photogenin.	Luciferin (photophelein).	Luciferase (photogenin).
Destruction temperature.	Boiling 1 to 5 minutes.	70 degrees or above (light disappears 52 to 56 degrees; returns on cooling).	Not destroyed by 10 minutes' boiling.	42 degrees	Above 70 degrees	60 degrees.
Distribution	Throughout *Cypridina* and many non-luminous forms.	Luminous organs of *Cypridina.*	Throughout firefly and many non-luminous forms.	Luminous organs of firefly.	Luminous organs of *Pholas.*	*Pholas* and many non-luminous forms.
Stability	Less stabile	More stabile	More stabile	Less stabile	More stabile	Less stabile.
Porcelain filtration	Positive	Positive	Positive		With difficulty	With difficulty.
Dialysis	Easily	Very slowly	Easily	Negative (?)	Slow	Negative.
Adsorption by bone-black and $Fe(OH)_3$.	Positive	Positive	Negative.			
Light with oxidising agents.	Negative	Negative	Negative	Negative	Positive	Negative.
Light with saponin, thymol, NaCl, etc.	Negative	Positive	Negative	Negative.		
Ether solubility	Negative	Negative	Negative	Negative	Negative	Negative.
Concentration just giving light.	1 to 1,700,000,000	1 to 1,700,000,000.				
Substance from one animal gives just visible light in—	25,600 c.c.	25,600 c.c.	3.2 c.c. for *Luciola parva.*	1.6 c.c. for *Luciola parva.*		
Effect of chloroform, ether, bensol, etc.	Fairly stabile	Stabile	Stabile	Quickly affected.	Very slowly affected.	Slowly affected.
Effect of alkalies	Not destroyed by n/30 NaOH in 1 hour.	Destroyed by n/125 NaOH in 1 hour.			Forms insoluble alkali-albumin with NH_4OH.	
	Reversible extinction of light by n/125 NaOH if neutralised immediately.					
Effect of acids	Destroyed by n/1000 HCl in 1 hour.	Destroyed by n/2000 HCl in 1 hour.			Not precipitated by acetic and H_2CO_3 except in presence of neutral salts.	
	Reversible extinction of light by n/2000 HCl if neutralised immediately.					

Effect of alcohols and acetone.	Reversible extinction of light with 20 per cent alcohol, 16 per cent acetone, and saturated butyl alcohol.		......	......	Precipitated by alcohol at 82° unchanged.	
Effect of tannic, picric and phosphotungstic acids.	Reversible extinction of light in n/500 to n/5000 concentration.		......	......	Precipitated by picric acid unchanged.	
Effect of KCN......	......No effect......		......No effect......			
Effect of NaF......	......	......	......	......	......	Not destroyed by 1 to 100.
Effect of enzymes....	......	......	......	......	......	Destroyed by trypsin.
Saturation with $(NH_4)_2SO_4$, NaCl, $MgSO_4$, sugar, and glycerine.	Reversible extinction except in case of acid $(NH_4)_2SO_4$.		......	......	Precipitated by $(NH_4)_2SO_4$; not by NaCl or $MgSO_4$; insoluble in glycerine.	Suspends activity and returns on dilution.
Color of light......	......Bluish white......		......Yellowish white......		Greenish blue	

between 45° and 54° and returns on cooling. The maximum temperature from which the light revived was 84° and it usually returned about 50°. These effects are similar to those observed with *Cypridina.*

Very weak concentrations of acids prevent the production of light of the firefly, less weak concentrations of alkalies are necessary, and KCN does not affect light-production in strong (m/100) concentrations.

Table 10 summarizes the characters of photogenin and photophelein (luciferase and luciferin) as found by Dubois for *Pholas* and by myself for *Cypridina* and the firefly. Where a statement runs through both photogenin and photophelein columns it applies to a mixture of the two substances. A blank indicates that the experiment has not been tried.

STUDIES ON A PENNATULID, CAVERNULARIA HABERI.

GENERAL CHARACTERS OF THE ANIMAL AND OF THE PHOTOGENIC MATERIAL.

Panceri (24), working with *Pennatula phosphorea,* has given us the best account of phosphorescence among the pennatulids. He describes the light as coming from eight bands of cells on the outer wall of the stomach and continued into buccal papillæ. They contain a luminous "fatty" matter which can easily be squeezed out as a sort of slime and which does not decompose readily. He also found that on stimulation a wave of light would pass over the colony in any direction at a rate of about 5 cm. per second. In a form like *Cavernularia* he describes also somewhat similar conditions (24, p. 40), but his descriptions are very meager. I have found the Japanese species of *Cavernularia* more favorable for light experimentation than *Pennatula,* and the following pages contain confirmation of Panceri's experiments on the light-waves passing over the colony and a general account of the chemistry of light-production.

Cavernularia haberi is especially abundant in the fjord of Aburatsubo, Japan, near Misaki, the marine station of the Imperial University of Tokyo. The colony of animals lies hidden in the sand and contracts during the day, but at night takes up water and expands, large ones to the length of 2 feet. If stimulated by touching or electrically, or by the addition of ammonia, a slime is formed similar to that produced by most of the *Cnidaria* upon irritation, but differing in that it is brightly luminous. The whole of the outer surface of the colony can form the luminous slime, but not the spongy inner material. The stalk, containing no polyps, is especially brilliant.

The slime may be dried over $CaCl_2$ and will give light when moistened with sea-water or fresh water.

By squeezing *Cavernularia,* from which most of the sea-water has been gently pressed, one can easily obtain a luminous juice which is still luminous when filtered through filter paper and retains its luminescence for several hours. Examination of the filtrate under the microscope in the dark shows that the light comes from minute points of light which make the field of view look like the starry heavens. If water is added to this sea-water juice, the light is greatly increased, due to the appearance of numerous additional points of light. These points of light come from minute granules and globules easily visible in the filtered juice under the microscope. The addition of water to a dark *Cavernularia* juice which has stood for two days will cause the appearance of light due to the dissolving of the granules and globules. So sensitive are they to the addition of water that 1 drop of fresh-water added to 5 c.c. of juice will produce light, and so bright is the light that the addition of 1 drop of *Cavernularia* juice to 5 c.c. of fresh-water produces a light easily visible when the drop is mixed with the 5 c.c.

By centrifuging the filtered juice, the granules may be partially thrown down and will give a brilliant light if fresh-water is added, while the liquid itself (turbid in appearance) gives a much faimter light. No light is produced upon the addition of water to a parchment-paper sea-water dialysate of the juice, so that light-production is undoubtedly connected with the visible globules and granules of the juice. That it is connected with the solution of the granules is indicated by the fact that fresh-water but not salt-water or isotonic cane-sugar is able to call forth the production of light. The process appears to be similar to the cytolysis of cells, as can be observed by an inspection of table 8, which shows the effect of adding various substances to the dark juice of *Cavernularia.*

The light-giving granules of *Cavernularia* will pass through an alundum filter crucible (R A 84) of the finest pores, but not through a Pasteur-Chamberland filter-tube. The liquid passing through the latter is perfectly clear and non-luminous and gives no light when water is added.

There is no adsorption of the light-producing substance by bone-black or $Fe(OH)_3$.

ELECTRICAL STIMULATION.

The juice of *Cavernularia* filtered through filter paper does not respond to the strongest interrupted induced shocks. The living colony, however, responds readily.

When a galvanic current is passed through one of the excised polyps mounted between non-polarizable electrodes, a flash of light occurs on the make and a series of flashes while the current is passing, which cease on the break. There is no flash of light on the break. A similar response can be observed with *Noctiluca* (2). It will be remembered that Romanes (25) observed a series of contractions in the bell of a medusa during the passage of a galvanic current, and the sartorius muscle of the frog often contracts on the make of a galvanic current, remains contracted during the passage of the current, and relaxes on the break.

If the whole colony be stimulated by weak induced shocks, there is a local production of light. There is usually no response to a single shock, but a ready response to three or more sent in in rapid succession.

With stronger stimuli, a wave of light, easily followed by the eye, passes over the colony in each direction from the point stimulated. With interrupted induced shocks, a series of waves of light follow one another in quick succession (not corresponding to the number of stimuli, however), reminding one of the series of electric shocks given out by the torpedo, only on a slower scale. The time-interval between separate flashes no doubt corresponds to the refractory period of the cells concerned.

On pressing deeply into the tissue and stimulating strongly, a much brighter light-response also results, which very slowly moves away from the point of stimulation and usually does not extend more than 2 or 3 cm. At the same time the whole colony contracts, the polyps are drawn in, and in this condition do not respond to electrical stimulation by light-production.

The wave of light above mentioned will pass in any direction over the colony and across a cut around the middle of the colony involving the whole of the external tissue. Some inner tissue must therefore be capable of conducting the stimulus.

LACK OF OXYGEN.

That the production of light by *Cavernularia* is an oxidation can be very readily determined by passing a current of hydrogen through the juice, when the light disappears, but promptly reappears upon admitting oxygen. A sample of phosphorescent juice kept 24 hours in an atmosphere of hydrogen gave light when air was admitted.

Considerable oxygen is necessary, as may be seen by keeping the juice in a tall test-tube, when it lights only at the surface in contact with air. The light appears through the tube, however, if mixed with air.

REDUCTASE, OXIDASE, AND CATALASE.

A tube of *Cavernularia* juice shut off from the air very quickly reduces (decolorizes) methylene blue. The blue color reappears when oxygen is admitted. This reaction has nothing to do with light-production, however, as it is given also by a non-luminous sea anemone (*Anthopleura xanthogrammica*) and many other animal tissues which can not produce light.

Unboiled *Cavernularia* juice also gives oxidase reactions upon addition of H_2O_2 with guaiac, pyrogallol (slight), *a*-napthol, para-phenylen diamine, indo-phenol reagent, ortol, and pyrocatechin. The boiled juice gave a slight positive test with guaiac, para-phenylen diamine and the indo-phenol reagent. Many other non-luminous tissues give these reactions, however, and there is abundant evidence to show that light-production in animals is not connected with the ordinary oxidases (peroxidases) (see p. 232).

As in all other organic tissues, catalase is also present in the juice of *Cavernularia.*

TEMPERATURE.

The juice of *Cavernularia* still gives a good light at 0° C., as do pieces of the colony if stimulated.

On raising the temperature, the light of *Cavernularia* juice disappears at 52° and does not reappear on cooling. Pieces of the colony slowly heated begin to light spontaneously at about 40°.

The light of *Noctiluca* (2) disappears at 48°, *Cypridina* at about 54°, a hydroid (*Sertularia* sp.) at 54°, the firefly (*Luciola*) at 42°, and luminous bacteria at 38°.

PHOTOGENIN AND PHOTOPHELEIN.

Unlike the firefly, *Cypridina*, and *Pholas dactylus*, it is impossible to separate the luminous juice of *Cavernularia* into two substances, photogenin and photophelein (luciferase and luciferin), one destroyed by boiling, the other not, which will give light when mixed. We can not, for instance, cause light to appear in *Cavernularia* juice which has stood until the light has disappeared by adding fresh luminous *Cavernularia* juice heated to boiling and then cooled; neither can we obtain light by adding juice heated to temperatures below boiling (88°, 81°, 71°, 61°, or 52° C.) and then cooled.

Another species of pennatulid, *Pennatula* sp., as also *Noctiluca* and the squid *Watasenia scintillans*, behaves as does *Cavernularia.* None of these organisms gives the photophelein-photogenin (luciferin-luciferase) reaction, for reasons at which we can only guess. The photogenin or photophelein may be, either of them, very unstabile, or there may be sufficient photophelein to use up all the photogenin. The evidence in this case seems to indicate that the photophelein is unstabile, as we can obtain a faint light with *Cypridina* photophelein or firefly photophelein (both prepared with sea-water) and the non-luminous *Cavernularia* juice (photogenin). *Pennatula* and *Noctiluca* photophelein gave negative results with *Cavernularia* juice, and *vice versa.*

TABLE 11.—*Effect of standing on light-producing power of Cavernularia juice.*

Juice + water	Bright light.
Juice 12 hours old at 20° C. + water	Bright light.
Juice 24 hours old at 20° C. + water	Fair light.
Juice 48 hours old at 20° C. + water	Faint light.
Juice 72 hours old at 20° C. + water	None.

The question may be asked, what substances are able to cause light to appear in the juice of *Cavernularia* which has stood until completely dark? Usually 4 to 6 hours are sufficient time for the juice to lose its luminescence. It is still capable of giving out a bright light if we add fresh-water (but not sea-water) to it, and it retains this potentiality for over two days at 20° C. (see table 11) and a shorter time at higher temperatures. As only fresh water and not sea-water will call forth the light, and as we know that light-production is connected with the granules of the juice, the process appears similar to the cytolysis of cells—*i. e.*, to the swelling and solution in fresh water of the granules which the cells contain. By this means something is liberated from the granules of *Cavernularia* juice which oxidizes with light-production. Table 12 gives the results of adding various other substances, pure or dissolved in sea-water, to the dark *Cavernularia* juice.

TABLE 12.—*Effect of substances in calling forth light from non-luminous Cavernularia juice.*

Solution or substance added.	Character of light.	Solution or substance added.	Character of light.
50 per cent sea-water	Bright light.	Ortol crystals	Faint light.
66 per cent sea-water	Fair light.	Hydrochinon crystals	None.
75 per cent sea-water	Fainter light.	Resorcin crystals	None.
80 per cent sea-water	Very faint light.	Pyrocatechin crystals	None.
90 per cent sea-water	None.	Pyrogallol crystals	None.
Water	Bright light.	Ba_2O_2 powder	None.
Sea-water	None.	Na_2O_2 powder	None.
Sea-water evaporated to one-half volume	None.	$KMnO_4$ crystals	None.
m cane-sugar	None.	$K_2Cr_2O_7$ crystals	None.
m/2 cane-sugar	Faint light.	$FeCl_3$	None.
m/4 cane-sugar	Fair light.	$FeSO_4$ crystals	None.
m/8 cane-sugar	Good light.	$K_4Fe(CN)_6$	Faint.
Chloroform-saturated sea-water	Faint light.	*Laonome japonica* (an annelid) blood	None. None.
One-half chloroform-saturated sea-water	Very faint light.	Dried ox-blood extract in sea-water	None.
One-quarter chloroform-saturated sea-water	None.	*Lepas anatifera* extract	None.
Chloretone-saturated sea-water	None.	*Lepas anatifera* extract, boiled	None.
One-half chloretone-saturated sea-water	None.	*Chiton* sp. extract	None.
One-quarter chloretone-saturated sea-water	None.	*Chiton* sp. extract boiled	None.
Thymol-saturated sea-water	Fair light.	*Onchidium* sp. extract (a pulmonate mollusk)	None.
One-half thymol-saturated sea-water	Faint light.	*Onchidium* sp. extract, boiled	None. None.
One-quarter thymol-saturated sea-water	Very faint light.	*Dolabella* sp. blood (a nudibranch)	Faint.
Benzol	Faint light.	*Dolabella* sp. blood, boiled	None.
Ether	None.	*Sepia esculenta* blood	None.
Chloroform	None.	*Sepia esculenta* blood, boiled	None.
Chloretone crystals	Very faint light.	*Panulirus japonica* blood	Very faint light.
Thymol crystals	Faint light.	*Panulirus japonica* blood, boiled	None.
Chloral-hydrate crystals	Faint light.	*Cypridina hilgendorfii* photophelein in sea-water	Fair light.
Saponin powder	Fair light.	*Luciola vitticollis* photophelein in sea-water	Fair light.
Oleic acid	Faint light.		

Note from table 12 that light-production is not due to dilution of the salts of sea-water by adding fresh-water, since m cane-sugar does not call forth the light. Note also that many cytolytic substances (chloroform, benzol, thymol, etc.) give light, but not the oxidizing agents, Na_2O_2, $KMnO_4$, etc. The blood of certain invertebrates also causes very faint light-production, but we can not be sure that this is not due to the fact that the blood is somewhat less concentrated than sea-water, although the determinations of other closely allied forms show the salt-content to be the same as the sea-water in which they live.

CHEMICAL REACTIONS.

The luminous juice of *Cavernularia* contains the luminous substance, but mixed with many substances, so that mere chemical tests on the juice are of no value in determining the chemical nature of the luminous substance. The luminous material is salted out along with the other proteins, but does not retain its power to phosphoresce long enough to be manipulated by the ordinary chemical methods. The following paragraphs give the results of precipitation by $(NH_4)_2SO_4$, picric acid, alcohol, etc.

If to the fresh filtered luminous juice of *Cavernularia* we add sugar, NaCl, $MgSO_4$, $(NH_4)_2SO_4$ to saturation, or 5 volumes of glycerine, the light disappears. A heavy precipitate forms in $(NH_4)_2SO_4$, a small precipitate in $MgSO_4$ and practically none in NaCl. The precipitates are soluble in sea-water. No precipitate is formed in sugar or glycerine. If poured into fresh-water or sea-water immediately after the light has disappeared, light is produced by all solutions. If the tubes are allowed to stand for a day, no light is produced by any tube on pouring into fresh-water or sea-water.

If alcohol or acetone (3 vols.) is added to the juice, a precipitate forms and the light disappears. The precipitate is insoluble and gives no light in sea-water or fresh-water.

The alkaloidal reagents—picric acid, phosphotungstic acid, and tannic acid—immediately cause precipitation with disappearance of light. The precipitates are insoluble in sea-water or fresh-water and produce no light.

Normal acetic acid or n NH_4OH, added drop by drop, produce no precipitate, although the light disappears in weak concentrations.

ANESTHETICS.

Ether, chloroform, and benzol cause a fairly rapid disappearance of the light in the order named, the ether most rapid. In turpentine, chloral hydrate, thymol, and chloretone the light stays for a long time and then disappears. There is no reappearance of light from any of the tubes if poured into fresh-water or sea-water unless we do so immediately the light has disappeared, and then the light is very faint and momentary.

In other words, the juice can not be anesthetized. Even in concentrations of alcohol, ether, chloroform, or butyl alcohol, which cause a slow disappearance of light, there is no recovery or only a momentary faint glow upon dilution of the solutions with sea-water. The alcohols give similar results. About 16 per cent ethyl alcohol will cause the light to disappear in one minute, but there is no recovery or only a very faint momentary glow on diluting with sea-water. Saturation with butyl alcohol gives a similar result.

Since reversibility is the *sine qua non* of anesthesia, we must conclude that this juice can not be anesthetized, in this respect differing markedly from the granule-free *Cypridina* luminous secretion which can be anesthetized by the alcohols. Also many luminous organisms—for instance, luminous bacteria—can be truly anesthetized (see p. 219).

POTASSIUM CYANIDE.

Potassium cyanide has no inhibiting effect upon the light-production of *Cavernularia* juice. Even in m/40 concentration the light is still bright after 90 minutes, a result agreeing with all other experiments on luminous organisms (bacteria, firefly, *Cypridina*, *Noctiluca*).

SUMMARY OF RESULTS FROM CAVERNULARIA.

1. The light of *Cavernularia* comes from a luminous slime, a secretion of gland-cells over the outer surface of the colony.

2. The secretion contains small granules which can be seen to emit the light. On standing the light from a sea-water extract of *Cavernularia* slowly disappears and will not return on shaking, but reappears if fresh water is added to the juice.

3. The light substance—*i. e.*, the granules—will pass an alundum filter (R A 84) but not a Chamberland filter. It is not adsorbed by boneblack or $Fe(OH)_3$ and will not dialyze.

4. The light-producing granules do not respond to electrical stimulation, but the colony gives light with galvanic or induced currents.

5. With galvanic currents a flash appears on the make and a series of flashes while the current is passing, which cease on the break.

6. Upon stronger stimulation (with induced currents) a wave of light passes over the colony in all directions from the point stimulated. This wave will pass through the deeper tissues when all the ectodermal tissues are cut.

7. A considerable amount of oxygen is used up in light-production and no light appears in its absence.

8. *Cavernularia* juice will decolorize (reduce) methylene blue in absence of oxygen, and it also contains peroxidases and catalase.

9. The light is still bright at 0° C. Fragments of *Cavernularia* give off light spontaneously at 40° C., which disappears at 52° C. and does not return on cooling.

10. The photogenin-photophelein reaction is not given by *Cavernularia*, but a faint light can be obtained with a non-luminous *Cavernularia* juice (photogenin) and *Cypridina* or firefly photophelein.

11. The production of light by the granules appears similar to the cytolysis of cells, as it occurs with water (but not isotonic cane-sugar) and certain cytolytic substances (saponin, chloroform, benzol, oleic acid).
12. The light-producing substances are salted out along with the proteins, but are not stabile enough for chemical manipulation.
13. The light-producing granules can not be anesthetized.
14. Potassium cyanide has no effect on light-production.

STUDIES ON LUMINOUS BACTERIA.

DESICCATION AND EXTRACTION WITH FAT SOLVENTS.

Luminous animals may be divided into two classes—those in which the luminous material is burnt within the living cell (firefly, fungi) and those in which it is secreted by the cell outside (many worms, crustacea, and myriapods). As first shown by Molish (8) the luminous substance must be burnt within the bacterial cell, since a dense emulsion of the luminous bacteria may be separated from the medium by a Chamberland or Berkefeld filter and a clear dark filtrate with no trace of phosphorescence obtained. I have repeated Molish's experiment and can confirm him. An alundum filter crucible was used. Can this photogenic substance in the cell be freed of other cell material and obtained in a more or less pure state? We know that the bacteria can be dried, and when moistened again will phosphoresce, even though the majority are not living, and will give rise to no new growth if inoculated in a suitable culture medium.[1]

These dried bacteria form the material used for extraction purposes. The organisms are best grown in bulk in a thin layer of peptone (1 per cent), glycerine (1 per cent), sea-water nutrient fluid covering the bottom of a white enameled pie-plate and covered by another pie-plate, the whole readily sterilized and serving as a large Petrie dish. The medium must be faintly alkaline to phenolphthalein. The bacteria are easily collected by centrifuging. The dense mass of centrifuged bacteria is then spread in a thin layer on filter paper or on glass wool, placed in a desiccator, and dried over $CaCl_2$ in a vacuum. Spreading on glass wool has the advantage that the glass wool may be ground up in a mortar and a powder obtained which phosphoresces when moistened, but the powder does not give so brilliant a light as does the filter paper containing dried bacteria. It is well to wash the glass wool with several changes of water to remove alkali. Such strips of dried bacteria-impregnated filter paper can be extracted with boiling ether or cold absolute alcohol for 12 hours without losing their power to phosphoresce when the solvent has been removed and they are again moistened. Colonies of luminous bacteria sometimes appear when the filter paper is placed on a nutrient medium, even after such rigorous treatment with ether and alcohol and other fat solvents. Büchner and Gaunt (26) obtained similar results with the acetic-acid-forming bacteria of beer (*Mycoderma aceti*). The dried acetic bacteria are not all killed by extraction with acetone; moist bacteria are invariably killed.[1]

---

[1] I have on two occasions obtained no luminous growths from bacteria which had been dried on filter paper and afterwards moistened (light was produced) with sterile sea-water and placed on nutrient agar culture medium. In the majority of cases, however, colonies of brilliantly luminous bacteria result. These colonies are relatively few in number, indicating that *most* of the bacteria are killed by drying.

In testing the solubility in fat solvents, strips of filter paper containing the dried bacteria were placed in the solvents in sterile tubes for a definite time at a definite temperature, the solvent completely removed, and the filter paper tested for light-production by adding sterile sea-water. A plus mark (+) indicates light, a minus mark (−) indicates no light. Controls, untreated with any solvent, always gave a good light. Table 13 gives the results. In the last column are similar results obtained with the dried powdered luminous organs of the firefly. The amyl alcohol and ethyl butyrate were completely removed by washing with ether.

The great majority of fat solvents extract nothing which is essential to light-production, as can be seen from the table. Chloroform might have extracted something, as the material glows only faintly after chloroform treatment. I have, however, evaporated the chloroform extract to dryness *in vacuo* and added water as well as a water extract of luminous bacteria (in itself non-luminous; possibly containing a second necessary substance) to the residue without obtaining light-production. The same result was obtained with the residue of the boiling alcohol extract, so that we must conclude that the chloroform and boiling alcohol extract nothing, but rather destroy the photogenic material. The temperature of boiling alcohol, 78.4°, is not destructive to the photogen. These results are very similar to my previous results on firefly material, as may be seen by inspecting the last column. The photogen of the firefly is not weakened by chloroform or acetone or a boiling mixture of equal parts alcohol and ether, but does suffer from carbon disulphide. Otherwise the results are the same.

The strips of filter paper moistened with sterile sea-water were then transferred under sterile conditions to nutrient agar to see if colonies of luminous bacteria would result. It was found that not in every case, but in at least one experiment out of several tried, luminous colonies were obtained after extraction of the material with ether, alcohol, toluol, acetone, and benzol.

I have already mentioned the fact that dried bacteria will glow if moistened after extraction with cold (20°) absolute alcohol, and also the fact that if 50 volumes of absolute alcohol are added to a mass of moist centrifuged bacteria and they are then shaken for 10 minutes, the alcohol removed, and bacteria quickly dried, no phosphorescence is obtained on moistening this dry powder. Neither will dried bacteria phosphoresce if extracted with 95, 80, 70, 50, or 37 per cent alcohol. What is the explanation of this? The alcohol does not dissolve out a luminous substance. Water appears to exert some influence. Perhaps we may explain the above result as merely an example of the effect of alcohol on dry albumins as opposed to albumin solutions. Powdered egg albumin can be extracted with absolute alcohol (or acetone) for 6 hours and is still readily soluble in water. But if a concentrated aqueous solution of albumen is precipitated by a large excess of alcohol (or acetone), even though the alcohol (or acetone) be removed within 15 minutes, the precipitate of albumen is found to be practically insoluble in water. It would be futile to discuss the matter without further experimental results, but the results seem to indicate that in photogenesis there is involved a substance which in the moist state is irreversibly precipitated (or changed) by alcohol and that it is probably protein in nature.

---

[1] I find that extraction of dried luminous bacteria with 95, 80, 50, or 35 per cent alcohol kills them all and no new growths appear. Also if the moist bacteria are treated with a large excess (50 volumes) of absolute alcohol or acetone for 10 minutes and then rapidly dried no growth is possible. Bacteria so treated have also lost their power to phosphoresce when moistened.

TABLE 13.

Solvent.	Temperature (in degrees C.).	Time of extraction (in hours).	Dried bacteria.	Dried firefly.
Ether (cold)	20	24	+	+
Ether (hot)	35	24	+	+
Chloroform (cold)	20	12	+	+
Chloroform (hot)	61	12	+ faint.	+
Ethyl alcohol (cold)	20	12	+	+
Ethyl alcohol (hot)	78.4	12	−	−
Alcohol and ether (equal parts, boiling).	46	12	− to faint light.	+
Acetone (cold)	20	12	− to faint light.	+
Acetone (hot)	56.3	12	−	+
Carbon tetrachloride	20	24	+	+
Carbon bisulphide	20	24	+ fair, light.	−
Toluol	20	12	+	+
Toluol (hot)	100	12	−	−
Benzol	20	12	+ fair.	Not tried.
Benzine (petroleum ether)	20	24	+ fair.	+
Amyl alcohol	20	24	+	+ faint.
Ethyl butyrate	20	24	−	−
95 per cent alcohol	20	24	−	+ very faint.
80 per cent alcohol	20	24	−	−
70 per cent alcohol	20	24	−	−
50 per cent alcohol	20	24	−	−
35 per cent alcohol	20	24	−	−
Material kept dry at	78.4	12	+ fair.	+
Material kept dry at	100	12	−	−

At any rate, the experiments show (1) that the photogenic material is not a fat or a fatlike body soluble in fat solvents, and (2) that phosphorescence does not depend on the living cell, since many of the dried bacteria which can still phosphoresce when moistened will give rise to no new colonies.

The question may now be raised as to whether bacteria whose structure has been completely destroyed by grinding in the dry state will phosphoresce on moistening. Oxidation can not take place so long as water is absent. Experiment shows that they will not phosphoresce, as the following procedure indicates: Luminous bacteria dried on glass wool are powdered in a porcelain mortar and divided into two equal parts, A and B. A was then ground in the porcelain mortar for 20 minutes with pure quartz sand; B was thoroughly mixed in another mortar with an equal volume of sand, previously ground for 20 minutes, and exposed to the air during the time of grinding A. In this way the effect of quartz powder or the possibility of absorbing moisture from the air would be the same for the unground bacteria B or the ground bacteria A. On moistening B with sterile sea-water, a good phosphorescence appeared, while the ground material gave no light with sea-water. I have repeated the experiment with the same result and feel that there are no possible sources of error. Microscopic examination shows the sand to be ground to the size of the bacteria or smaller and it is well known that even the smallest cells may be broken up by grinding with sand. I find that the dried luminous organs of the firefly likewise lose their power to phosphoresce if thoroughly ground with sand. This result differs from that of McDermott (21), who finds that firefly tissue can be frozen and ground in liquid air without losing its power to phosphoresce. Both A and B were inoculated on agar nutrient medium. The ground bacteria, A, gave rise to no luminous colonies, while the unground bacteria, B, did develop several luminous colonies—further proof that the ground bacteria were wholly broken up and destroyed.

EFFECT OF LACK OF OXYGEN.

Many observers have shown that if luminous bacteria are suspended in sea-water and the oxygen removed the light disappears and again returns when oxygen is readmitted. Will the light reappear if the cells are in the meantime cytolyzed? I have made many attempts to obtain extracts of cells broken up in absence of oxygen which would give light if oxygen were readmitted. All these efforts have failed. The result might have been anticipated by the work of MacFadyen (27), who found that luminous bacteria subjected to the action of liquid air did not phosphoresce at that low temperature, but did phosphoresce as soon as warmed again; further, that if the cells were broken up by grinding at the temperature of liquid air, there was no phosphorescence on rewarming. MacFadyen worked, however, in the presence of oxygen and moisture, and we might suppose that a slow oxidation—too slow to produce light—went on in the material broken up at low temperatures with consequent exhaustion of the photogenic material.

In my experiments the moist bacteria have been broken up (cytolyzed) in absence of oxygen by (1) oxygen-free distilled water and (2) toluol. All marine cells can be cytolyzed by distilled water or fat solvents.

In the first method a dense mass of bacteria are placed in a vessel from which the air is exhausted by an air-pump (see apparatus, p. 195). The bacteria stop glowing, but reglow if air is again admitted. Then oxygen-free distilled water is allowed to flow onto the bacterial mass and it is thoroughly shaken. No light appears (indicating that the water is oxygen-free), and 5 to 10 minutes later, if oxygen is added, still no light is emitted. If there is a definite soluble photogenic substance in the bacterial cell it should have passed into solution in the water when the cell was cytolyzed, and, provided no decomposition took place, it should have glowed when oxygen was readmitted. Even if we assume that the cell was not completely cytolyzed, the photogen, if a stable substance, although one unable to pass the cell surface, should have glowed within the cell.

In the second method a dense emulsion of the bacteria in sea-water is rendered non-luminous by removing the oxygen. Then a drop of toluol is added without admitting oxygen (air). The emulsion is shaken and no light appears. In a few minutes air is admitted and still no light appears. Similar experiments with ether, chloroform, and carbon tetrachloride gave similar results. Thus if the cells are broken up the photogen disappears even though it has not been oxidized, for no oxygen was present. The toluol itself does not destroy the photogenic substance, as evidenced by the treatment of dried bacteria with toluol. Luminous bacteria in oxygen-containing sea-water to which a drop of toluol, ether, chloroform, or carbon tetrachloride is added very quickly stop phosphorescing. I explain this as due to the fact that on cytolysis of the cell the oxidation processes run riot and the available store of photogen is rapidly used up. The same explanation may be applied to the loss of light in distilled water. We may compare the conditions in bacteria to the conditions in a potato cell. When the cells of the potato are crushed or when their surface is destroyed by toluol or ether or chloroform, dark melanin oxidation products are rapidly formed, but if the potato is cut and the cut cells well washed to free them of their cell-contents, no blackening occurs, although the lower intact cells at the cut surface are exposed to atmospheric oxygen and only separated from it by their plasma membranes. A destruction of these membranes would immediately cause oxidations within to proceed rapidly.

The conclusion drawn from the above experiments has been confirmed by allowing oxygen-free sea-water to come in contact with dried bacteria in a hydrogen atmosphere. If, after 15 minutes, oxygen is admitted, no glow is observed, although dried bacteria instantly glow for a short time if moistened with oxygenated sea-water.

All the above experiments, then, point to the conclusion that if the cell is broken up while moist or if the dead cells stand in contact with water for any length of time, even though no oxygen be present, nevertheless the photogenic substance undergoes decomposition, a conclusion in harmony with my work and that of McDermott on the firefly. As we have seen, extraction of the dried firefly luminous organs with oxygen-free solvents will give no phosphorescent solutions on admitting oxygen, because of this instability of the photogen (p. 196). On the other hand, the substances in *Cypridina* extract are stable in oxygen-free water (see p. 176).

In the normal living bacterial cell (or firefly cell) I assume the photogen to decompose through oxidation with light-production. If the living bacteria are kept in sea-water from which all oxygen has been removed and they stop glowing, they will still glow strongly if oxygen is readmitted, even after a period of 24 hours. It is therefore obvious that the breaking-up of the photogen in absence of oxygen does not occur in the intact bacteria, but only in those whose normal "structure" has been destroyed by cytolysis. I am inclined to believe that the surface layer of the cell is the "structure" involved.

PHOTOGENIN AND PHOTOPHELEIN.

As we have just seen, the light of a mass of bacteria disappears almost instantly if they are broken up by any of the usual cytolytic agents. This result is no doubt due to the fact that they continually burn their light-producing substances as soon as they manufacture them. The substances are therefore present in very small quantity. For this reason we might expect to fail in demonstrating the existence of photogenin and photophelein, although we must remember that forms like *Cavernularia*, which contain a large amount of luminous material, also fail in showing the photogenin-photophelein reaction.

Since it is practically impossible to grind the luminous bacteria in sea-water and so prepare an extract of photogenin, we are forced to prepare our extract by cytolyzing with distilled water and thus obtain a dark solution containing swollen suspended bacteria. This fluid will give no light, however, on adding a suspension of bacteria heated to boiling. Thinking that bacterial photophelein as well as photogenin might be destroyed by boiling temperature, I tried heating to lower temperatures, 90°, 80°, 70°, 60°, and 50°, for 2 minutes, but in no case did light appear on mixing with photogenin. Neither did these solutions give light on mixing with firefly photogenin, but we can obtain light from a bacterial photophelein prepared in the following way: by adding absolute alcohol to a dense mass of the bacteria, then removing the alcohol by centrifuging, and quickly drying by evaporation *in vacuo*. The resultant powder gives no light with water, but does cause firefly photogenin to phosphoresce very faintly. Similar material prepared with acetone instead of alcohol gave no light with firefly photogenin. In order to obtain a bacterial powder which will give light with firefly photogenin it is necessary to remove the alcohol and dry as quickly as possible; otherwise the photophelein is destroyed.

Addition of ether to a mass of luminous bacteria causes the light to disappear rapidly and it is possible that the photogenin is destroyed by ether and not the photophelein; but bacteria so treated, after the ether had been evaporated by a current of air, gave no light with firefly photogenin. Negative results were obtained also with an alkaline (m/12 KOH) extract of luminous bacteria neutralized with HCl. A precipitate of nucleoprotein (?) is produced on neutralization. Apparently the bacterial photophelein is formed in such small quantities from its precursor or is so unstable that all methods of obtaining it have failed, with the exception of the rapid precipitation by alcohol.

TABLE 14.

Organism.	Observer.	Minimum.	Maximum.
		° *C.*	° *C.*
Bacterium phosphorescens....	Lehmann...	−12	39.5
Bacterium phosphoreum......	Molisch....	−5	28
Light bacteria...............	Tarchanoff..	−7	37–50

TEMPERATURE.

The temperatures shown in table 14 have been recorded for the extinction of the light of luminous bacteria (28). The variations in the results suggest that further observations were desirable. Accordingly luminous bacteria, isolated from fish, were grown on absorbent cotton saturated with beef-broth-peptone-glycerol culture medium. Free access of air between the cotton fibers supplies the conditions for a brilliant light and at the same time an excellent means of handling the bacteria. A wisp of cotton strongly phosphorescent with bacteria was placed in a very thin-walled glass tube about 2 mm. in diameter and attached to a thermometer bulb as for melting-point determinations. On slowly raising the temperature, the light (to a dark-accustomed eye) becomes dim at 30°, very dim at 34°, and disappears at 38°. On slowly lowering the temperature the light weakens at 0°, is very dim at −7°, and disappears at −11.5°. These values agree best with those given by Lehmann for *Bacterium phosphorescens* and do not greatly exceed the usual temperature limits of activity of organisms.

Bacteria raised to 38°, and then cooled, phosphoresce only very dimly, but, as first observed by MacFadyen (27), again glow brilliantly at room temperature even after an exposure to liquid air.

OXIDASE AND CATALASE.

Many enzymes are found in bacteria in a condition which defies extraction except by means of the Büchner press, and it is difficult to obtain luminous bacteria in sufficient quantity for this method. The oxidases seem to be contained in these bacteria in this endoenzyme condition. All my efforts to obtain an oxidase in solution which will oxidize the common oxidase reagents—guaiac, α-napthol, para-phenylen diamine, phenolphthalin, and pyrogallol—have failed, even though hydrogen peroxide is added.

Similar results have been obtained by other workers. Gessard (29) showed that a melanogenic variety of *Bacterium pyocyaneum* would turn tyrosin brown, but he was unable to separate a solution of tyrosinase from the bacteria. Lehmann and Sano (30) found also that *Actinomyces chromogenes*, *Bacterium putidum*, and *B. phosphorescens* would oxidize tyrosin in the culture medium, but *Vibrio indicus*, which phosphoresces strongly, *Sarcina lutea*, *B. typhi*, *B. coli*, and many others would not. They found in addition that a substance oxidizing aloin and giving a very weak guaiac reaction could be extracted from *Actinomyces chromogenes*, *B. putidum*, and *B. phosphorescens* by a mixture of two parts glycerine to one of water, but no tyrosinase reaction could be obtained with this extract. Here also the tyrosinase is apparently in an endoenzyme condition.

As we shall see (p. 225) the vegetable oxidases are capable of oxidizing pyrogallol with light-production, but many attempts to produce light with pyrogallol and extracts of luminous bacteria failed.

Catalase also occurs in the luminous bacteria with which I worked, but in small amount.

LUMINOUS BACTERIA AND OSMOTIC PRESSURE.

Most marine animals require an external medium of some definite osmotic pressure, and they will not live below this limit; some marine forms (*Fundulus*) are independent of an external osmotic pressure. Luminous bacteria belong to the first category, as indicated in table 15. They will not live in dilute sea-water, but they will live in it if its osmotic pressure is raised by some inert substance like isotonic cane-sugar. Note also that some salt is necessary for the continued production of light, as the bacteria no longer glow after 24 hours' immersion in m sugar, a fact of no great surprise, as unicellular fresh-water luminous animals are unknown.

In these experiments, as well as those with the salts of sea-water, acid and alkalies, and alcohols, one drop of a dense emulsion of luminous bacteria (a form isolated from squid at Woods Hole, Massachusetts) was added to 30 c.c. of solution in an uncorked Erlenmeyer flask and the whole thoroughly mixed. For comparative observations it is essential that the eye be thoroughly adapted to the dark and that each flask be oxygenated by shaking, before judging as to the emission or absence of light. Observations were made after 10 minutes, 1 hour, and 24 hours.

TABLE 15.—*Effect of dilution of sea-water with water and with m cane-sugar solution.*

Dilution with water.					Dilution with m cane-sugar.				
Parts sea-water.	Parts water.	Light after—			Parts sea-water.	Parts m sugar.	Light after—		
		10 min.	1 hour.	24 hours.			10min.	1 hour.	24 hours.
2	1	+	+	+	2	1	+	+	+
1	1	Faint.	Faint.	−	1	1	+	+	+
1	2	Faint.	Very faint.	−	1	2	+	+	+
1	4	Faint.	−	−	1	4	+	+	Faint.
1	6	Very faint.	−	−	1	6	+	+	Faint.
1	10	−	−	−	1	10	+	+	Faint.
1	14	−	−	−	1	14	+	+	−
1	20	−	−	−	1	20	+	+	−
Sea-water.....		+	+	+	m cane-sugar...		+	+	−
Water.......		−	−	−					

LUMINOUS BACTERIA AND BALANCED SOLUTIONS.

Not only must luminous bacteria be bathed with a certain concentration of salts, but any salt of sea-water is not sufficient. It must be a particular salt, although luminous bacteria, as indicated in table 16, are not so affected by the pure monovalent cations of sea-water as are most cells.

TABLE 16.—*Effect of various combinations of the salts of sea-water.*

Salt combinations.	Light after—		
	10 min.	1 hour.	24 hours.
Sea-water..................................	+	+	+
Artificial sea-water, m/2(100 $NaCl$+2.2 KCl +2 $CaCl_2$+ 10 $MgCl_2$) +n/4000 $NaOH$...	+	+	+
Neutral artificial sea-water................	+	+	Faint.
m/2 $NaCl$..........................	+	+	Faint.
m/2 KCl..........................	+	Faint.	−
m/3 $CaCl_2$..........................	−	−	−
m/3 $MgCl_2$..........................	−	−	−
m/2(100 $NaCl$+2.2 KCl).................	+	+	Faint.
m/2(100 $NaCl$+2 $CaCl_2$).................	+	+	Faint.
m/2(100 $NaCl$+10 $MgCl_2$).................	+	+	Faint.
m/2(100 $NaCl$+2.2 KCl+2 $CaCl_2$)........	+	+	Faint.
m/2(100 $NaCl$+2.2 KCl+10 $MgCl_2$).......	+	+	Faint.
m/2(100 $NaCl$+2 $CaCl_2$+10 $MgCl_2$).......	+	+	Faint.

Note the independence of these bacteria of a balanced medium. The bacteria live and phosphoresce in pure NaCl without the addition of any bivalent ions. This is true even when the solution is changed three times to remove the last traces of Ca in the solution. KCl is also relatively non-toxic, although more so than NaCl. $CaCl_2$ and $MgCl_2$ are very toxic when alone. All combinations of NaCl with the other ions of sea-water sustain the bacteria well, except that they are neutral media, and hence the phosphorescence is dimmed after 24 hours. That pure NaCl should have so little effect on light-production is astonishing when we consider its poisonous effect on other marine organisms and tissues, particularly on ciliated cells.

TABLE 17.—*Effect of acid and alkali.*

Concentration of acid and alkali added to Mg-free sea-water, m/2(100 NaCl+2.2 KCl+2 $CaCl_2$) in Syracuse watch glasses.	Light after—		
	10 min.	1 hour.	24 hours.
n/2000 HCl	−	−	−
n/4000 HCl	Faint.	−	−
n/8000 HCl	+	Faint.	−
n/16000 HCl	+	+	−
n/32000 HCl	+	+	Faint.
n/500 valerianic acid	−	−	−
n/1000 valerianic acid	Faint.	−	−
n/2000 valerianic acid	Faint.	−	−
n/4000 valerianic acid	+	Faint.	−
n/8000 valerianic acid	+	+	−
n/16000 valerianic acid	+	+	Faint.
n/500 NaOH	−	−	−
n/1000 NaOH	−	−	Faint.
n/2000 NaOH	+	+	+
n/250 methyl amine	−	−	−
n/500 methyl amine	Faint.	−	Faint.
n/1000 methyl amine	Faint.	+	Faint.
n/2000 methyl amine	+	+	+
Mg-free sea-water	+	+	Faint.
Sea-water	+	+	+

EFFECT OF ACIDS AND ALKALIES.

As NaOH precipitates the Mg of an artificial sea-water, it is always necessary to use Mg-free sea-water in order that the concentration of NaOH may not be reduced. The acids were also added to a neutral artificial Mg-free sea-water as well as the alkalies, in order to obtain comparative results. Table 17 gives the results. The return of light in n/1000 NaOH and n/500 methyl amine after 24 hours was possibly due to neutralization of alkali through absorption of CO_2.

As was to be expected, acids and alkalies were found to prevent light emission in very weak concentration, the acids in much weaker concentration than the alkalies. In fact, the bacteria are very sensitive to acid and will not even phosphoresce with any brilliancy in a neutral medium.

The organic acid (valerianic) and alkali (methyl amine) have less effect than the inorganic, a result at variance with my results for other organisms which are usually affected more readily by the weak than by the strong acids and alkalies (12 and 13).

EFFECT OF HOMOLOGOUS ALCOHOLS (ANESTHETICS).

As is indicated in table 18, the effect of the alcohols on light-production is very similar to their effect on other life processes; they exert an inhibiting or anesthetic action which is perfectly reversible. If alcohol solutions containing bacteria which have stopped emitting light are diluted with sea-water, light-production again begins. As with other tissues, the higher the alcohol in the series the greater anesthetic power it has.

TABLE 18.—*Effect of alcohols.*

Concentration of alcohol added to sea-water.	Light after—		
	10 min.	1 hour.	24 hours.
Methyl alcohol, 2m	–	–	–
HCH_2OH, 1.5 m	+	Very faint.	–
HCH_2OH, m	+	Faint.	–
HCH_2OH, m/2	+	+	+
HCH_2OH, m/3	+	+	+
Ethyl alcohol, m	–	–	–
CH_3CH_2OH, m/1.5	Very faint.	–	–
CH_3CH_2OH, m/2	+	Faint.	Faint.
CH_3CH_2OH, m/3	+	+	+
Propyl alcohol, m/3	–	–	–
$CH_3CH_2CH_2OH$, m/4	Very faint.	–	–
$CH_3CH_2CH_2OH$, m/6	Faint.	Very faint.	–
$CH_3CH_2CH_2OH$, m/8	+	Faint.	–
$CH_3CH_2CH_2OH$, m/15	+	+	+
Isobutyl alcohol, m/10	–	–	–
$(CH_3)_2CHCH_2OH$, m/12	Very faint.	–	–
$(CH_3)_2CHCH_2OH$, m/16	+	Very faint.	–
$(CH_3)_2CHCH_2OH$, m/20	+	+	–
$(CH_3)_2CHCH_2OH$, m/24	+	+	+
Amyl alcohol, m/20	–	–	–
$C_2H_5CH_3CHCH_2OH$, m/40	–	Very faint.[1]	+[1]
$C_2H_5CH_3CHCH_2OH$, m/80	Very faint.	Very faint.[1]	+[1]
$C_2H_5CH_3CHCH_2OH$, m/160	Faint.	+[1]	+
$C_2H_5CH_3CHCH_2OH$, m/320	+	+	+
Capryl alcohol, m/400	–	–	–
$CH_3(CH_2)_5CH_2OH$, m/800	Very faint.	Very faint.	–
$CH_3(CH_2)_6CH_2OH$, m/1600	Faint.	Faint.	–
$CH_3(CH_2)_6CH_2OH$, m/3200	Faint.	Faint.	+[1]
$CH_3(CH_2)_6CH_2OH$, m/6400	+	+	+
Sea-water	+	+	+

[1] Probably due to evaporation of alcohol.

The effect of a number of other substances was studied in a very rough way, namely, by adding a small quantity of the substance to a sea-water emulsion of the bacteria in test-tubes and then shaking the tubes. With toluol, benzol, ether, chloroform, carbon disulphide, carbon tetrachloride, and ethyl butyrate the light was found to disappear almost immediately; with tannin, chloral hydrate, vanillin, and sodium glycocholate the light had disappeared in the course of an hour, while saponin, amygdalin, and sodium taurocholate had no effect. It is surprising that saponin has no effect on luminous bacteria when we consider its great cytolytic power on other forms in very small concentration.

EFFECT OF POTASSIUM CYANIDE.

As is the case with other luminous forms, KCN has a surprisingly small effect on the light-production of luminous bacteria as compared with its effect in supressing certain oxidations of many animals. The results are expressed in table 19.

TABLE 19.—*Effect of potassium cyanide.*

Concentration of KCN in sea-water.	Light after—		
	10 min.	1 hour.	24 hours.
m/40	−	−	−
m/80	Faint.	Very, very faint.	−
m/160	Faint.	Very faint.	−
m/320	Fair.	Fair.	−
m/640	+	+	−
m/1280	+	+	−
m/2560	+	+	+
Sea-water	+	+	+

In m/40 KCN the light disappears in 6 minutes, and if the solution is now diluted with sea-water the light returns. The effect is therefore an inhibition and reversible.

SUMMARY OF RESULTS FROM LUMINOUS BACTERIA.

1. Luminous bacteria which have been rapidly dried over calcium chloride in a vacuum will phosphoresce if moistened with oxygen-free water. Drying does not kill all bacteria, but does kill most of them. Hence phosphorescence does not depend on the living cell.

2. Dried bacteria, if finely ground with sand, will no longer phosphoresce when moistened. None of the ground bacteria can grow. Phosphorescence does depend upon the integrity of some structure in the cell.

3. Dried bacteria extracted with ether or toluol will still phosphoresce if moistened and may develop colonies on a suitable culture medium. Consequently neither ether nor toluol destroy the photogen.

Bacteria in oxygenated sea-water to which ether or toluol is added stop phosphorescing, presumably because the photogenic substance is rapidly oxidized and used up when the bacterial cell is cytolyzed.

Bacteria in oxygen-free sea-water do not glow, but will glow if oxygen is admitted, even after a period of 24 hours. Bacteria in oxygen-free sea-water to which toluol or ether is added will not glow if oxygen is readmitted after 15 minutes. Hence the phosphorescent substance undergoes decomposition in the absence of oxygen, a decomposition not due to the toluol (compare the first statement in section 3), but probably due to the action of some other substance.

4. Moist luminous bacteria to which oxygenated distilled water is added cease glowing, presumably because the photogen is rapidly oxidized and used up when the bacterial cell is cytolyzed.

Moist bacteria to which oxygen-free distilled water is added will not glow even momentarily if oxygen be readmitted after 15 minutes, a result again pointing to instability of the photogen when the cell-structure is affected by cytolysis.

5. Dried bacteria placed in oxygenated sea-water phosphoresce momentarily, but if dried bacteria stand in contact with oxygen-free sea-water for 15 minutes, no phosphorescence occurs when the oxygen is admitted. Again (as in sections 3 and 4) the photogen has decomposed. It is, therefore, impossible to extract a phosphorescent substance from bacteria with oxygen-free aqueous solvents.

6. Fat solvents extract nothing which will phosphoresce from the dried bacteria. Some of the bacteria survive and will grow after such extraction. Boiling alcohol, cold acetone, and ethyl butyrate destroy the power to phosphoresce.

7. Dried bacteria do not lose their power to phosphoresce after 24 hours' extraction with cold absolute alcohol, but moist bacteria (centrifuged) treated with 50 volumes of absolute alcohol, and then dried rapidly, will not again phosphoresce if moistened.

8. All attempts to separate the photogenic substance of luminous bacteria into photogenin and photophelein have failed, due possibly to the small amounts of these substances present at any one time.

9. A photophelein can be prepared by absolute alcohol which will give a faint light with firefly photogenin, but attempts to obtain light with firefly photophelein and bacterial photogenin prepared in various ways have failed.

10. The upper temperature-limit for luminescence is 38°; the lower is −11.5°. Bacteria heated to 38° and cooled give only a faint light, but bacteria will glow strongly if first cooled to liquid-air temperature and then raised to room temperature.

11. Oxidases for guaiac, a-napthol, para-phenylen diamine, phenol, phenolphthalin, pyrogallol, or indo-phenol formers, if present, are present in an endoenzyme condition. Catalase exists in luminous bacteria.

12. Luminous bacteria can not live if the osmotic pressure of the medium fall below a certain value or if the salt-content of the medium fall below a certain value.

13. Luminous bacteria will live for over 24 hours in pure m/2 NaCl or any sea-water salt mixtures containing both monovalent and divalent cations. They will live for over an hour in m/2 KCl, but the light disappears instantly in m/3 $CaCl_2$ or m/3 $MgCl_2$.

14. Luminous bacteria will just live for slightly over an hour in n/8,000 HCl, n/4,000 valerianic acid, and will live for over 24 hours in n/1000 NaOH and n/500 methyl amine. The best light is given on the alkaline side of neutrality, about the alkalinity of sea-water.

15. The homologous aliphatic monohydric alcohols produce true reversible inhibition or anesthesia of light-production. The concentrations which inhibit light-production in 10 minutes are: methyl alcohol, 2 m; ethyl, m; propyl, 0.25 m; isobutyl, 0.08 m; amyl, 0.025 m; capryl or octyl, 0.002 m.

16. KCN has very little effect on light-production. The light is only slightly affected in m/320 concentration in one hour.

STUDIES ON A SQUID, WATASENIA SCINTILLANS.

GENERAL CHARACTERISTICS OF LIGHT-PRODUCTION.

The structure of the luminous organs and the habits of this form have been well described by Ishikawa (31) and Sasaki (32). The organs are found on the tip of the ventral arms (3 in number), on the eyeball (5 in number), and over the ventral surface of the body (many). Only those of the arm-tips are brilliant enough to make chemical studies feasible. Their light is a brilliant bluish white like that of *Cypridina*. The intensity is high, but is no doubt due in large part to the reflectors present in the gland. The luminous material is burned within the cells. The animal is a deep-sea form, coming to the surface near shore to breed in certain bays of the Japanese coast, notably Toyama Bay. The surface-water of this bay is brackish and the animals will not live in it for even one hour. The deeper waters of the bay are more salty and in these the squid will live for a longer time if the water is changed to remove the poisonous ink. If oxygen be forced through fine pores into the water, the squid, as Professor Shoji, of Kyoto University, has shown, will live perhaps 8 hours, but this is the maximum. It will be seen, therefore, that the animal is extremely delicate and it is not surprising to find it of little value for chemical research. The arm-tip light-organs respond only to stimulation and the response disappears in 5 to 10 minutes after the animals are removed from sea-water. If the arms are cut off and placed in sea-water the light-producing substance entirely disappears in 30 to 60 minutes, as we can determine by grinding in a mortar. No light appears. The arm-tip light-organs become exhausted very rapidly if continually stimulated, and the light disappears very quickly after the organs have been ground in a mortar with water or sea-water.

The light of the skin-organs scattered over the ventral surface lasts much longer, and even after removal from the sea-water for 1 hour they will give a light which is more yellowish if stimulated electrically. The response is localized in the region about the electrodes.

The eye-organs are difficult to stimulate. Their light is weak and similar to that of the skin-organs.

The squid are caught at night in enormous numbers during April, May, and June by the fishermen, and Professor C. Ishikawa informs me that if brought into a dark room in the daytime they give light. The squid for this experiment were caught at 3 a. m. and tested about 8 a. m.—*i. e.*, 3 or 4 hours after daylight had appeared. There is no inhibiting influence of daylight on light-production, as described by Peters (4) for *Ctenophores* or Moore (5) for various luminous plankton forms. Neither have I observed any such influence of light with *Monocentris*, *Cypridina*, *Cavernularia*, *Pennatula*, or *Noctiluca*.

PHOTOGENIN AND PHOTOPHELEIN.

An extract presumably containing *Watasenia* photogenin can be prepared in the usual way by grinding the arm-tip light-organs in a mortar. As already mentioned, the light fades very rapidly, and it is impossible to again obtain light by mixture with the boiled-tissue extract of the squid (or with tissues heated to temperatures below boiling), which presumably should contain photophelein. Many attempts were made to obtain the photogenin-photophelein reaction, but all failed. This result is probably due to the fact that *Watasenia* contains more photophelein than photogenin, so that all the photogenin is used up when the arm-tip organs are ground. At least, *Watasenia* contains photophelein which will give light with firefly photogenin, whereas the firefly photophelein will give no light with *Watasenia* extracts which we might suppose to contain photogenin (p. 198).

STUDIES ON A FISH, MONOCENTRIS JAPONICA.

Monocentris japonica is a shallow-water marine fish having two light-organs—one on each side of the under surface of the tip of the lower jaw. The light is a weak glow with the faintest bluish tinge and is continuous and of almost unvarying intensity. In this respect it differs from most fishes, whose light is a series of flashes, the result of stimulation (Oshima, 33). The steady glow of the organs of *Monocentris* can be observed if the fish is removed from daylight to a dark room. The structure of the organ has never been investigated.

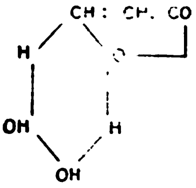
If the organ is ground in a mortar, the light lasts for some time, but does not reappear if mixed with *Monocentris* photophelein. Neither was light obtained by mixing *Monocentris* photogenin and *Cypridina* photophelein or *vice versa*, but as material was scarce, only one experiment was performed, and these results must not be considered conclusive.

STUDIES ON OXIDATIVE CHEMILUMINESCENT REACTIONS WITH SUBSTANCES OF DEFINITE COMPOSITION.

HISTORICAL.

Since the researches of Radziszewski in 1877 (34) and 1880 (35) on lophin and various oils, alcohols, and aldehydes, we have come to recognize an ever-increasing number of substances which will luminesce under appropriate conditions at temperatures approaching those compatible with life. Lophin (triphenylglyoxaline) gives an especially good light with alcoholic potash at 60° C., as do many essential oils. Trautz (36) has added other substances to the list of chemiluminescent reactions. R. Dubois (37) discovered the luminescence of esculin, a glucoside obtained from horse-chestnut bark and chemically a combination of glucose and esculetin.

These substances all require fairly strong alkali, although I have a sample of bergamot oil which gives a beautiful light when mixed with n/10 NaOH at 20° C. and a faint light at 5° C. At 20° C. a faint light appears with n/80 NaOH. On warming, the light is brighter. n/80 NaOH is, however, incompatible with life. Lophin and esculin will not luminesce except with alcoholic NaOH or KOH.



Esculetin.

McDermott (38) has described the production of light if to urine is added some 99 per cent KCN or potassium formate or formaldehyde and then a strong alkaline solution of H_2O_2. The substance in urine responsible for this effect is unknown. He describes light-production upon adding strong alkaline H_2O_2 to the cleavage products of Witte's peptone or glue made with alkali in absence of oxygen and to which a little commercial formaldehyde is added. (Note that the light is connected in these cases with substances found in organisms.)

Ville and Derrien (39) made an interesting step forward when they showed that lophin would luminesce with blood and H_2O_2, and Dubois (10) has recently discovered that esculin also will give light with blood and H_2O_2. The blood contains an oxygen carrier, hemoglobin, which accelerates the oxidation of the lophin and esculin. I can confirm the statement of Dubois and of Ville and Derrien, and find, also, contrary to Dubois, that esculin will give light with $KMnO_4$ and H_2O_2. It is best to keep the temperature about 60° C. I found also that esculin would give light with $FeCl_3+H_2O_2$, but not with $FeCl_3$, or $KMnO_4$ or H_2O_2 alone, nor with plant extracts rich in oxidases (turnip, potato, and horseradish root), either with or without H_2O_2, and that blood extract still retains its power to produce light with lophin after boiling. This is not surprising, as blood will also oxidize guaiac after boiling and upon addition of H_2O_2. Ozonized turpentine can take the place of H_2O_2 in oxidation of esculin.

Trautz (36) has shown that if we mix 35 c.c. of a 50 per cent K_2CO_3 solution, 35 c.c. of 10 per cent pyrogallol, and 35 c.c. of 35 per cent formaldehyde, and to this mixture add 50 c.c. of 30 per cent H_2O_2, a glow occurs, accompanied by much foaming. I can confirm this result, and find in addition that when the glow has died, if we add some $KMnO_4$, a reddish glow again appears. The tube becomes perceptibly warm.

It is not necessary to use such strong reagents to obtain light from oxidation of pyrogallol, however, for a very weak solution of pyrogallol will give a bright light if oxidized under the proper conditions. The oxidation of a mixture of pyrogallol + H_2O_2 by the vegetable oxidases occurs with the production of light. The reaction is highly interesting and remarkable for the following reasons: Perceptible light is produced with the concentration of pyrogallol m/32,000—*i. e.*, 1 part in 254,000 parts of solution; a faint light is produced at 0° C. and a bright light at 10° C.; KCN inhibits the reaction in m/2,000 concentration; boiling destroys the oxidase, and the power of producing light just as boiling destroys the light-producing power of organisms.

THE CHEMILUMINESCENCE OF PYROGALLOL.

It has been known for a long time that pyrogallol takes up oxygen from the air in presence of alkali and is converted into brown oxidation products of unknown composition. The depth of brown coloration (and consequently the extent of oxidation) depends on the concentration of alkali added. I have never under any conditions obtained light during this oxidation, although concentrations of alkali ranging from n to n/6,000 NaOH, both with and without H_2O_2, have been used. It has also been long known that pyrogallol will turn brown with H_2O_2 and some oxidizing enzyme from organisms. Purpurogallin, a substance of doubtful composition ($C_{11}H_8O_5$?), is said to be formed and the reaction has been used in studying oxidizing enzymes quantitatively. Only if H_2O_2 be present will this reaction produce light. It is similar to the oxidation of gum guaiac to guaiacum blue, but I find that guaiacum blue can be formed under many conditions when pyrogallol can not be oxidized with light-production. Thus hydrogen peroxide is necessary for light-production, even when potato-juice is used as the oxidase solution—*i. e.*, even when a juice is used containing an oxidase which will oxidize guaiac tincture directly (without addition of H_2O_2). It is then strictly a peroxidase which is responsible.[1] Blood also gives the reaction if H_2O_2 is present. Catalase takes no part, and may be destroyed by heating the potato-juice to 60°.

---

[1] The difference between potato-juice, which oxidised guaiac without addition of H_2O_2, and turnip-juice, which requires the addition of H_2O_2, appears to lie in the fact that the potato-juice contains a substance which oxidizes spontaneously in presence of oxygen to a peroxide, while the turnip-juice does not. Consequently, we must add a peroxide to the turnip-juice. This spontaneously oxidizable substance was called oxygenase by Bach and Chodat. Both juices contain peroxidase. The "direct oxidases" (of potato) consist, then, of peroxide (oxygenase) + peroxidase, while the "indirect oxidases" (of turnip) are peroxidase alone. In this paper oxidase is used as a general name for an oxidizing enzyme and peroxidase for an enzyme transferring oxygen from a peroxide to an oxidizable substance. (See the account of this subject in Bayliss's Principles of General Physiol., 1915, 584; also see the monographs of Kastle (42), Warburg (Ergeb. d. Physiol., XIV, 253, 1914), Batelli and Stern (Ergeb. d. Physiol., XII, 96, 1912), and Bach and Chodat in Abderhalden's "Biochemische Arbeitsmethoden, III, No. 1, p. 42. Literature lists are given in the first three monographs.)

Thinking that this reaction might throw some light on luminescence in organisms, I have studied it in some detail, with especial reference to the substances which are known to affect light-production in organisms. It is the oxidases, similar to those which blue guaiac, that are concerned in the light-production of pyrogallol and potato-juice, but, as we shall see, many inorganic catalyses are also able to oxidize pyrogallol with light-production. It may be noted in passing that although light can be produced at 0° C., a considerable amount of heat is given off, and in this respect the oxidation differs markedly from those giving light in organisms.

TABLE 20.

Concentration of pyrogallol in a mixture which contains (after mixing) 50 per cent potato-juice and 0.93 per cent H_2O_2.	Character of light from the mixture.	Concentration of pyrogallol in a mixture which contains (after mixing) 0.93 per cent H_2O_2 and 0.5 per cent blood-extract.	Character of light from the mixture.
m/4	Faint.	m/40	Faint.
m/8	Fair.	m/80 to m/8000	Bright.
m/16 to m/8192	Bright.	m/16000	Fair.
m/16384	Fair, faint.	m/32000	Faint.
m/32768	Very faint.	m/64000	Very faint, none.
m/65536	None.	m/128000	None.

In studying the reaction I have used mostly pure potato-juice (*i. e.*, the pressed, unfiltered extract of grated potatoes, strained through cheese-cloth and decanted from the starch-grains) or a 1 per cent ox-blood extract (*i. e.*, 1 gram of dried defibrinated ox blood to 99 parts water) added to an equal volume of a half-and-half mixture of m/100 pyrogallol and 3 per cent H_2O_2 (Merck's perhydrol). The experiments will be given in the form of tables or data under the following heads:

1. Concentration of the three substances necessary for light-production.
2. Substances taking the place of blood or potato oxidase, pyrogallol, and H_2O_2.
3. Effect of temperature on the reaction.
4. Effect of KCN, NaOH, and HCl.
5. Effect of fat-solvent anesthetics.
6. Is the oxidase a catalyzer?

1. CONCENTRATION OF THE THREE SUBSTANCES NECESSARY FOR LIGHT-PRODUCTION.

In general, the more concentrated the blood or potato-juice the brighter the light, and the more concentrated the pyrogallol (to a limit) the brighter the light, and the longer it lasts. Too strong pyrogallol precipitates the blood-extract and potato-juice, the former in weaker concentration than the latter (see table 20). In about m/8000 concentration the pyrogallol gives a bright light with potato-juice, and increasing the concentration above this does not increase perceptibly the brightness.

Each substance must be present in a definite small concentration to produce light. This is m/32,000 for pyrogallol, 0.025 per cent for H_2O_2, 0.1 per cent for dried blood, and 5 per cent for potato-juice—*i. e.*, 5 parts pure potato-juice to 95 parts water.

Note also, from tables 21 and 22, that very often the guaiac reaction can be obtained when, under similar conditions, no light-production by pyrogallol takes place.

TABLE 21.

Concentration of potato-juice in a mixture containing (after mixing) m/400 pyrogallol and 0.75 per cent H_2O_2.	Character of light from the mixture.	Blue color with tincture of guaiac.	Concentration of blood-extract in a mixture containing (after mixing) m/400 pyrogallol and 0.75 per cent H_2O_2.	Character of light from the mixture.	Blue color with tincture of guaiac
25 per cent.....	Bright.....	Blue.	0.5 per cent.....	Bright.....	Blue.
10 per cent.....	Faint......	Blue.	0.25 per cent....	Faint......	Blue.
5 per cent.....	Very faint..	Faint blue.	0.05 per cent....	Very faint..	Blue.
2.5 per cent....	None......	Faint blue.	0.025 per cent...	None......	Blue.
0.5 per cent....	None......	Very faint blue.	0.01 per cent....	None......	Blue.

2. SUBSTANCES TAKING THE PLACE OF OXIDASE, PYROGALLOL, AND H_2O_2.

Oxidase.—Any plant extract containing strong oxidases, such as occur in turnips, sweet potatoes, horseradish, etc., or any blood containing hemoglobin, will give light with pyrogallol and H_2O_2. Apple-juice, which oxidizes tannin, gives no light. Extracts of many invertebrates were tried (including insects, molluscs, annelids, crustaceans, and echinoderms), but only those of *Chiton*, a tubicolous annelid (*Laonome japonica*), and some polychætes have thus far been found to give light. The extract must be concentrated and the light-production is probably due to hemoglobin, as it is obtained in many cases after the extract is boiled. The substances in *Chiton* seem to come from red filaments near the mouth (muscles ?). Contrary to my earlier experiments, which were performed with too-dilute extracts, I find that the blood of a squid (*Sepia esculenta*) (containing hemocyanin) and the orange blood of the Japanese lobster (*Panulirus japonica*), will also give light, and the light is somewhat brighter if the blood has previously been boiled. The extracts of luminous animals never gave light with pyrogallol+H_2O_2. Among these, bacteria, an annelid (*Chætopterus*), a squid (*Watasenia scintillans*), a crustacean (*Cypridina*), a protozoan (*Noctiluca*), a pennatulid (*Cavernularia*), and the firefly have been tried.

Inorganic substances which give light may be divided into three classes: (1) insoluble precipitates, (2) colloidal metals, (3) solutions of various salts. H_2O_2 must always be added. The first two classes probably act as true inorganic catalyzers.

Among the substances of class (1) may be mentioned MnO_2, mixtures of $Mn(OH)_2$ and $Mn(OH)_3$, H_2MnO_3, $Fe_2Fe(CN)_6$, and Ag_2O. All give light. I have observed no light with boneblack, PbO_2, Ag_2CrO_4, $PbCrO_4$, $BaCrO_4$, $Ag_4Fe(CN)_6$, $Pb_2Fe(CN)_6$, $Zn_2Fe(CN)_6$, $Cu_2Fe(CN)_6$, HgO, $Cu(OH)_2$, Cu_2O, CuO, FeO, iron filings, Hg_2Cl_2, Cr_2O_3, $Cr(OH)_3$, $Fe(OH)_3$, Pt black, and silica.

Among the group (2), a silver sol protected by protein so as to be fairly concentrated gave a good light. Concentrated Pt sol and Au sol also gave light. Colloidal $Fe(OH)_3$ and copper oxide gave negative results.

TABLE 22.

Concentration of Merck's special H_2O_2 in a mixture containing (after mixing) m/400 pyrogallol and 50 per cent potato-juice.	Character of light from the mixture.	Blue color with tincture of guaiac.
0.47 per cent	Bright	Blue.
0.23 per cent	Fair	Blue.
0.11 per cent	Weak	Blue.
0.05 per cent	Faint	Blue.
0.025 per cent	Very faint	Blue.
0.012 per cent	None	Blue.

The following salts gave the best light in the concentrations indicated: m/10 to m/20 $K_4Fe(CN)_6$, m/100 $K_2Cr_2O_7$, m/50 K_2CrO_4 (the light appears only after a long time), m/100 $KMnO_4$, m/100 CrO_3, m/10 $FeCl_3$. The following gave no light: $K_2S_2O_8$ (m/10 to m/320), $KClO_3$ (m/10), $K_3Fe(CN)_6$ (m/10 to m/1000) $MnCl_2$ (m/10), $AgNO_3$ (2 per cent), $CuSO_4$ (m/10), and bromine water. The mechanism of the action of these diverse substances will be considered in a subsequent paper.

Peroxide.—Only H_2O_2 will give light with pyrogallol and an oxidase. Na_2O_2, benzoyl hydrogen peroxide, quinone, BaO_2, or ozonized turpentine do not, although they give a strong guaiac reaction with the peroxidase of turnip-juice. We must also add H_2O_2 to potato-juice, which already contains an organic peroxide whose oxygen may be transferred to guaiac. It is no doubt in too small a concentration to give light with pyrogallol.

Oxidizable substance.—Many of the amino- and hydroxy-phenols are rapidly oxidized by potato-juice + H_2O_2, but pyrogallol was the only one which gave light. The following were tried and all gave colored oxidation products: phenol, tricresol, pyrocatechin, resorcin, hydrochinon, orcinol, *a*-napthol, ortol, anilin, *o* and *p* toluidin, *a*-napthylamin, guaiacol, para-phenylen diamine, amidol, and pyramidon. Phloroglucin, *B*-napthol, and *B*-napthylamin were not oxidized.

Among the glucosides, vanillin, amygdalin, phloridizin, salicin, saponin, and esculin gave no light with potato-juice, either at 20° or 60° C.

3. Effect of Temperature on the Reaction.

A very faint light is produced when pyrogallol + H_2O_2 at 0° C. is mixed with potato-juice at 0° C. The light is much brighter at 5° and quite bright at 10° C. Pyrogallol + H_2O_2 mixed with blood (1 per cent) gives no light at 0° C., but a very faint light at 2° C.

High temperatures destroy the pyrogallol oxidase of potato-juice. This occurs between 80° and 85° C., as indicated in table 23. The potato-juice was rapidly heated to the temperatures 50°, 55°, 60° C., etc., then cooled rapidly in running water. The destruction of catalase, the formation of a precipitate in the potato-juice, and the effect on its power to blue guaiac alone, guaiac + H_2O_2, and guaiac + ionized turpentine, are also recorded. Similar results were obtained with turnip-juice. Blood does not lose its power to oxidize pyrogallol + H_2O_2 with light-production or guaiac + H_2O_2, even after 10 minutes' boiling.

TABLE 23.

Potato-juice heated to—	Light-production with pyrogallol + H_2O_2.	Bluing of guaiac.	Bluing of guaiac + H_2O_2.	Bluing of guaiac + ozonized turpentine	Effect on catalase; liberation of O from H_2O_2.	Formation of precipitate in potato-juice.
50 degrees	Bright	Blue	Blue	Blue	+++	None.
55 degrees	Bright	Blue	Blue	Blue	+	None.
60 degrees	Bright	Blue	Blue	Blue	None	None.
70 degrees	Faint	Slight bluing.	Fair bluing.	Blue	None	Precipitate.
80 degrees	Very, very faint.	Very slight bluing.	Slight bluing.	Blue	None	Precipitate.
85 degrees	None	None	None	Blue	None	Precipitate.
90 degrees	None	None	None	Blue	None	Precipitate.
100 degrees	None	None	None	Slight	None	Precipitate.
100 deg. (10 min.)	None	None	None	None	None	Precipitate.

Note from table 23 that the pyrogallol and guaiac oxidases in presence of H_2O_2 are both destroyed between 80° and 85° C., but that with ozonized turpentine the juice heated to 100° C. still blues guaiac faintly. The natural peroxide (oxygenase) of potato-juice, which disappears on standing, is destroyed more readily than the peroxidase. Catalase is destroyed between 55° and 60° C. before a precipitate has appeared, whereas peroxidase is still active after a precipitate has appeared.

4. Effect of KCN, NaOH, and HCl.

In testing the effect of these substances, equal parts of (1 c.c. m/160 pyrogallol + 1 c.c. 3 per cent H_2O_2) and (potato-juice + KCN or NaOH or HCl) were mixed. Table 24 gives also the effect of catalase on the bluing of guaiac + H_2O_2, and the color of indicators added to determine the amount of acid or alkali uncombined with protein. Neutral red is red in neutral or acid solution, yellow in alkalies. "Di-methyl" is yellow in neutral or alkaline solution, red in free acid solution.

The concentration of acid and alkali added give, of course, no idea of the H-ion concentration in the potato-juice. The oxidase acts best in the region of neutrality or slight alkalinity, and Reed (40) finds by hydrogen electrode measurements that the oxidase of potato is inhibited in a $C_H 1.5 \times 10^{-4}$. The effect of the acid is reversible unless it has acted too long.

Note from table 24 that KCN affects the pyrogallol peroxidase in very weak concentration (m/2,000), but is not so effective in destroying catalase or preventing the bluing of guaiac. NaOH and HCl are not so toxic. As we might expect, the HCl is the more toxic of the two.

TABLE 24.

Substance.	Concentration in potato-juice.	Light-production with pyrogallol $+ H_2O_2$.	Liberation of O from H_2O_2.	Bluing of guaiac $+ H_2O_2$.	Color of indicator.	Indicator.
KCN	m/20	None	+	None	Yellow	Neutral red.
	m/40	None	+	Very faint blue.	Orange	
	m/80	None	++	Fair blue	Orange	
	m/160	None	++	Fair blue	Red	
	m/320	None	++	Fair blue	Red	
	m/640	None	++	Fair blue	Red	
	m/1280	None	+++	Blue	Red	
	m/2560	Very faint	+++	Blue	Red	
	m/5120	Faint	+++	Blue	Red	
	m/10240	Fair	+++	Blue	Red	
	m/20480	Bright	+++	Blue	Red	
NaOH	m/20	None	+	None	Yellow.	
	m/40	None	+++	Faint blue	Yellow.	
	m/80	Faint	+++	Blue	Yellow orange.	
	m/160	Bright	+++	Blue	Red orange.	
	m/320	Bright	+++	Blue	Red.	
HCl	m/20	None	None	None	Red orange	Dimethylamino asobenzol.
	m/40	None	None	Faint blue	Yellow	
	m/80	None	None	Faint blue	Yellow	
	m/160	Fair	+	Blue	Yellow	
	m/320	Bright	+++	Blue	Yellow	
	None	Bright	+++	Blue	Yellow	

5. EFFECT OF FAT-SOLVENT ANESTHETICS.

Saturation of the potato-juice or blood with ether or chloroform does not affect its power to produce light with pyrogallol $+ H_2O_2$. A sample of horseradish-root extract preserved 45 days under ether gave a good light when mixed with pyrogallol $+ H_2O_2$.

6. IS THE OXIDASE A CATALYZER?

That oxidases in general are not catalyzers, but are used up in the reaction has been proven by the work of Kastle (16), who found that the amount of phenolphthalein oxidized is proportional to the quantity of blood present, and of Bach and Chodat (41 and 42), who found that the amount of pyrogallol oxidized to purpurogallin is proportional to the amount of peroxidase and also to the amount of H_2O_2 present, but independent of the quantity of pyrogallol, providing the pyrogallol is present in excess of the quantity capable of being oxidized by peroxidase and H_2O_2. Melanin formation by tyrosinase is also proportional to the amount of tyrosinase present (42).

The peroxidase, therefore, transfers the O of H_2O_2 to the pyrogallol and the H_2O_2 pyrogallol, and peroxidase are all changed—*i. e.*, used up in the reaction. If we represent the peroxidase by P and the pyrogallol by B the reaction must take place as follows:

$$\begin{aligned} P + 2H_2O_2 &= PO_2 + 2H_2O \\ PO_2 + B &= PO + BO \end{aligned} \qquad (1)$$

and not as

$$\begin{aligned} P + 2H_2O &= PO_2 + 2H_2O \\ PO_2 + 2B &= P + 2BO \end{aligned} \qquad (2)$$

Note that in the first equations the peroxidase (P) is used up and in the second equations it acts as a true catalyzer and is regenerated again.

That the peroxidase of turnip-juice is used up so far as its power to cause light-production in pyrogallol is concerned is indicated in the following experiment:

If 1 c.c m/10 pyrogallol + 4 c.c. H_2O_2 (3 per cent) is mixed with 5 c.c. turnip-juice heated to 80° (to destroy the catalase and weaken the peroxidase), a very faint light is produced. A small amount of peroxidase is, therefore, present. The mixture is allowed to stand for 24 hours at 22° C. and then fresh turnip-juice is added. A good light results, showing that the pyrogallol has not been changed by a small amount of peroxidase when a long time has been allowed for the reaction to proceed. The pyrogallol was turned a light brown by the small amount of peroxidase, and this color had not deepened in 24 hours, but did deepen immediately when the additional quantity of peroxidase was added. A small quantity of "enzyme" can not, therefore, transform an indefinite amount of pyrogallol. A similar result was obtained with a weak hemoglobin solution, 0.1 per cent dried blood-extract.

That a large quantity of peroxidase can be used up by addition of successive amounts of pyrogallol—*i. e.*, by "titrating" the peroxidase—can be shown as follows:

To 10 c.c. potato-juice heated to 60° C. (to destroy catalase and consequent foaming due to liberation of O from H_2O_2) is added 2 c.c. of a mixture of equal parts m/10 pyrogallol and 3 per cent H_2O_2. Light results. When this has disappeared (about 2 to 3 minutes) 2 more cubic centimeters of the pyrogallol-H_2O_2 mixture are added and a very faint light results. On adding a further 2 c.c. no light appears. There is, however, in the final mixture plenty of pyrogallol + H_2O_2 to produce light, as may be shown by adding fresh potato-juice when abundant light appears. The peroxidase has been completely used up. A similar experiment with blood-extract gave a similar result.

SIGNIFICANCE FOR BIOLUMINESCENCE.

The reader has doubtless already noticed the similarity of the pyrogallol+H_2O_2-peroxidase reaction and the luciferin-luciferase reaction of Dubois. Just as Dubois supposed luciferin to be oxidized by luciferase, so we actually find pyrogallol+H_2O_2 oxidized by peroxidase. We now know that luciferin (photophelein) is not oxidized by luciferase (photogenin) but that Dubois's luciferase is actually the *source* of the light of luminous animals; so that the luminescence of pyrogallol turns out to bear only a superficial resemblance to that of organisms. Pyrogallol+H_2O_2 will give no light with either photogenin or photophelein, nor will peroxidase give light with either photogenin or photophelein, even if we add H_2O_2. However, it is interesting and important to know that certain substances found in plants and animals can assist in oxidizing other substances found in plants and animals with the production of light. The light produced in these cases is faint compared with the light produced by luminous organisms or compared with the light produced by substances from luminous organisms in a test-tube, but nevertheless it is light with very little heat, and perhaps a further survey will reveal the existence of definite compounds producing light in a manner more closely resembling that of *Cypridina*. As has been shown by Trautz (36) the spectrum of luminescent pyrogallol is continuous but shorter than the normal spectrum, so that there is marked similarity in the physical characteristics of the light of chemiluminescent substances and of luminous organisms.

SUMMARY OF STUDY OF CHEMILUMINESCENT REACTIONS.

1. Pyrogallol gives off a yellowish-white light about equal in intensity to a suspension of luminous bacteria when oxidized by blood or plant-juices (oxidases) in presence of H_2O_2.

2. The light is visible in very weak concentrations of pyrogallol, viz, m/32,000 or 1 part to 254,000 parts solution. H_2O_2 must be present in at least 0.025 per cent, blood in 0.1 per cent (dried defibrinated blood), potato-juice in 5 per cent (fresh pure juice), to give a just visible light.

3. Of many easily oxidizable hydroxy- and amino-phenols only pyrogallol gives light. $K_4Fe(CN)_6$, $KMnO_4$ or $FeCl_3$, $K_2Cr_2O_7$, K_2CrO_4, MnO_2, $Mn(OH)_2+Mn(OH)_3$, H_2MnO_3, $Fe_2Fe(CN)_6$, Ag_2O, and colloidal Pt, Au, and Ag will all give light with pyrogallol. Extracts of invertebrates, except those of *Chiton*, a few marine annelids, and the blood of a squid (*Sepia*) and a marine crayfish (*Panulirus*), do not give light. Na_2O_2, BaO_2, PbO_2, benzoyl H_2O_2, quinone, or ozonized turpentine will not take the place of H_2O_2.

4. Light is produced at 0° C. and a bright light at 10° C. The oxidase of plant-juices is destroyed between 80° and 85° C.

5. KCN inhibits the light-production in very weak concentration, m/1280 to m/2560. NaOH inhibits in n/40 and HCl in n/80 concentration, but these figures give no idea of the H-ion concentration.

6. Ether and chloroform have no effect on the light-production.
7. The oxidase is not a true catalyzer, but is used up in transferring oxygen from H_2O_2 to the pyrogallol.
8. The reaction has nothing to do with the production of light by luminous animals.

GENERAL CONCLUSIONS.

We may be very certain that in all luminous animals a definite substance undergoes chemical change, and if free oxygen is present light is produced. This oxidizable substance may conveniently be called photogenin. In some forms it is oxidized within the cell, in others without. In some forms the oxidation goes on continuously, in others only after stimulation, using the word "stimulation" in the same sense in which it is used in referring to muscle contraction. In some forms photogenin occurs as granules, in others it may be obtained in a solution which will pass the finest porcelain filters. In some forms we can demonstrate the existence of a second substance necessary for light-production, photophelein; in others this can not be demonstrated. There are definite quantitative relations between photogenin and photophelein, and in those animals in which the two substances can not be demonstrated they are possibly present in equivalent amount. Photogenin is colloidal and probably a protein; photophelein is crystalloidal and of wholly unknown composition. Neither of the two are soluble in fat solvents. Photogenin is found only in luminous glands, photophelein throughout the organism and in non-luminous animals. Photopheleins from different species of luminous or non-luminous animals will give light with various photogenins if the animals are nearly related, but only a very faint light if distantly related. The connection between photogenin and photophelein resembles most that between the zymase (=photogenin) and cozymase (=photophelein) of yeast-juice. Both of the former substances must be present for the fermentation of sugar by yeast, just as both of the latter must be present for the production of light by luminous animals. As regards destruction by heat and dialyzability even the properties of the two corresponding substances are similar. Here the parallel ceases, at least so far as our knowledge is concerned. It is possible that a mixture of photogenin and photophelein oxidizes a third substance, just as zymase-cozymase oxidizes sugar, but we know of no third oxidizable substance. We do know that whenever photogenin and photophelein in solution are exposed to free oxygen, light is produced and both substances disappear. This is the present extent of our knowledge and only additional experimental work can give us a more definite idea of the nature of these substances.

BIBLIOGRAPHY.

(1) Harvey, E. N. 1913. Biochem. Bull., ii, p. 456.
———. 1914. Science, n. s., xl, p. 33.
———. 1915. Amer. Jour. Physiol., xxxvii, p. 230; Jour. Amer. Chem. Soc., xxxvii, p. 396; Biol. Bull., xxix, p. 30.
———. 1916. Science, n. s., xliv, p. 208; xliv, p. 652; Amer. Jour. Physiol., xli, p. 449, p. 454.
———. 1917. Amer. Jour. Physiol., xlii, p. 318, p. 342, p. 349.
(2) Harvey, E. B. 1917. Carnegie Inst. Wash. Pub. No. 251.
(3) Lund, E. J. 1911. Jour. Exper. Zool., xi, p. 415.
(4) Peters. 1905. Jour. Exp. Zool., ii, p. 103.
(5) Moore, B. 1909. Biochem. Jour., iv, p. 18.
(6) Phipson, T. L. 1876. Chem. News, xxxii, p. 22.
(7) ———. 1876. Jour. Frank. Inst., ci, p. 68.
(8) Molish, H. 1904. Leuchtende Pflanzen.
(9) Dubois, R. 1885. Compt. Rend. Soc. Biol., xxxvii, p. 559.
(10) ———. 1913 and 1914. Ann. d. la Soc. Linn. de Lyon, lx and lxi.
(11) Loeb, J. 1907. Pfluger Arch., cxviii, p. 36; 1908, cxxii, p. 196; 1908, cxxiv, p. 37.
(12) Harvey, E. N. 1914. Intern. Z. f. Physik-Chem. Biol., i, p. 463.
(13) ———. 1911. Jour. Exp. Zool., x, p. 507.
(14) Seidell, A. Solubilities of Inorganic and Organic Substances.
(15) Bayliss, W. M. 1915. Principles of General Physiology, pp. 221–222.
(16) Kastle, J. H. 1909. U. S. Pub. Health and Mar. Hosp. Serv. Bull. 51, p. 30.
(17) Harden and Young. 1906. Proc. Roy. Soc., B. lxxvii, p. 405; lxxviii, p. 369.
(18) Townsend, A. B. 1904. Amer. Nat., xxxviii, p. 127.
(19) Dahlgren, U. 1915, 1916, 1917. Jour. Frank. Inst.
(20) McDermott, F. A. 1911. Jour. Amer. Chem. Soc., xxxiii, p. 1791.
(21) ———. 1911. Smithsonian Report, p. 345.
(22) Dubois, R. 1912. Eighth Intern. Cong. App. Chem., xix, p. 86.
(23) McDermott, F. A. 1915. Jour. Amer. Chem. Soc., xxxvii, p. 403.
(24) Panceri. 1872. Ann. de Sc. Nat., 5th ser., xvi, pp. 13 and 40.
(25) Romanes. Phil. Trans. Roy. Soc., clxvi, clxvii, and clxxi.
(26) Büchner and Gaunt. 1906. Liebig's Annalen, cccxlix, p. 140.
(27) MacFadyen. 1902. Proc. Roy. Soc., B. lxxi, p. 76.
(28) Mangold. Article in Winterstein's "Vergleichende Physiologie," iii, pt. 2, p. 345.
(29) Gessard. 1901. Ann. de l'Inst. Pasteur, xv, p. 817.
(30) Lehman and Sano. 1908. Arch. f. Hygiene, lxvii, p. 99.
(31) Ishikawa, C. 1913. Zool. Anz., xliii, p. 162.
(32) Sasaki, M. 1914. Jour. Coll. Agric. Sapporo, vi, p. 75.
(33) Oshima, H. 1911. Jour. Coll. Sci. Tokio, xxvii.
(34) Radziszewski, B. 1877. Ber. d. d. Chem. Gesellsch., x, p. 70.
(35) ———. 1880. Leibig's Ann. d. Chem., cciii, p. 305.
(36) Trautz, M. 1904. Zeitschr. f. Electrochem., x, p. 593; 1905, Zeitschr. phys. Chem., liii, p. 1.
(37) Dubois, R. 1901. Comp. Rend. Acad. Sci., cxxxii, p. 431.
(38) McDermott, F. A. 1913. Jour. Amer. Chem. Soc., xxxv, p. 824.
(39) Ville and Derrien. 1913. Comp. Rend. Acad. Sci., clvi, p. 2021.
(40) Reed, G. B. 1916. Jour. Biol. Chem., xxvii, p. 299.
(41) Bach and Chodat. 1908. Biederman's Centralbl. f. agrik. Chem., xxxvii, p. 168.
(42) Kastle, J. H. Hyg. Lab. Bull. No. 59, Washington, pp. 80 and 112.
(43) Vogel, R. 1915. Zeit. wiss. Zool., cxi.
(44) Bongardt, J. 1903. Zeit. wiss. Zool., lxxv.
(45) Geipel, E. 1915. Zeit. wiss. Zool., cxii.
(46) Williams, F. X. 1916. Jour. Morph., xxviii, pp. 145.

IX.

A PHYSIOLOGICAL STUDY OF SPECIFIC GRAVITY AND OF LUMINESCENCE IN NOCTILUCA, WITH SPECIAL REFERENCE TO ANESTHESIA.

BY ETHEL BROWNE HARVEY,
Formerly of Cornell University Medical School, New York City.

A PHYSIOLOGICAL STUDY OF SPECIFIC GRAVITY AND OF LUMINESCENCE IN NOCTILUCA, WITH SPECIAL REFERENCE TO ANESTHESIA.

By Ethel Browne Harvey.

INTRODUCTION.

The present study has been chiefly concerned with the specific gravity and the effects of anesthetics on the luminescence of *Noctiluca*. The effects of other substances—acids, alkalies, KCN, etc.—have also been studied, as well as the action of electricity, change of osmotic pressure, temperature, etc., in order to gain an accurate knowledge of the more general physiological aspects of luminescence in this form. Some observations on these subjects were made by Quatrefages in 1850, Krukenberg in 1887, and Massart in 1893, but most of the work is too old and the methods too crude to be of much value, since definite concentrations were not considered nor the results interpreted from the physico-chemical aspect.

The results were obtained at the Marine Biological Laboratory of the Imperial University of Tokyo, situated at Misaki, Japan, where the great abundance of *Noctiluca* offered unusual advantages. The animals occur in such quantities that a layer an inch thick on the surface of a large aquarium jar can soon be collected, and on dark nights the sea is extremely brilliant in any area where it is disturbed, especially where the waves lap the shore. It gives me pleasure to thank Professor I. Ijima for the facilities of the laboratory, and also Professor C. Ishikawa for his many kindnesses during my stay there.

LIGHT RESPONSE IN NOCTILUCA.

As is well known, noctilucas, when undisturbed and under perfectly normal conditions, are not luminous; but when disturbed or stimulated in any way, they give a bright flash of a distinctly bluish tinge, lasting only an instant. This is the normal light-response of *Noctiluca*. If, however, the animals are not in good condition, a faint, continuous glow issues from them; such a glow is usually preliminary to the death of the animals, and is also called forth by many experimental conditions. These two are the only light responses of the animals. There is never, under normal conditions, a rhythmic flashing, such as is characteristic of certain species of firefly, nor is it possible, by any chemical or physical means, to call forth a rhythmic flashing comparable with the rhythmic twitching of a muscle in pure NaCl.

CHANGE OF OSMOTIC PRESSURE.

Noctiluca can stand a considerable decrease in osmotic pressure without having its luminous response affected, as indicated in table 1. We may dilute the sea-water with fresh-water in any amount down to a proportion of half and half, and the animals still give a normal response—*i. e.*, they flash on stimulation. The response is not quite so bright as in the control in a half-and-half concentration nor in a mixture of 4 sea-water to 6 fresh-water, but the animals continue giving a normal response for 7 days. In the latter concentration the animals also give a steady glow for the first few minutes, owing doubtless to the dying condition of certain of them. If the amount of fresh water is still further increased, the animals do not flash on stimulation, but give a steady glow, lasting for 8 minutes in a mixture of 3 sea-water to 7 fresh-water and of 2 sea-water to 8 fresh-water; and for 5 minutes in 1 sea-water to 9 fresh-water, and for about 3 minutes in pure fresh-water. When the osmotic pressure is increased by concentrating the sea-water to half its volume, a steady glow is given for more than 20 minutes.

TABLE 1.—*Effect of diluting sea-water with fresh-water.*

Ratio of sea-water to fresh-water.	Luminous response.	Specific gravity.
9 to 1	Normal, 10 days (experiment discontinued).	Float.
8 to 2	Normal, 10 days (experiment discontinued).	Float.
7 to 3	Normal, 9 days	Float.
6 to 4	Normal, 9 days	Distributed through water 10 minutes; then float.
5 to 5	Normal, 7 days (poor)	Most sink in 15 minutes; float after 20 minutes.
4 to 6	Normal, 7 days (poor), constant glow 3 minutes at start.[1]	Sink in 15 minutes; float after 1 hour.
3 to 7	Constant glow 8 minutes	Sink.
2 to 8	Constant glow 8 minutes	Sink.
1 to 9	Constant glow 5 minutes	Sink.
Fresh-water.	Constant glow 3 minutes	Sink.

[1] The constant glow at the start is without doubt due to the dying condition of some of the animals.

Especially interesting results have been obtained in this series of experiments with regard to specific gravity. Normally, noctilucas are less dense than sea-water, so that when placed in an aquarium jar they soon rise to the top, where they form a layer somewhat pinkish in color. Unlike the floating siphonophores, there are no air-bubbles, and unlike certain pelagic eggs, there are no large oil-drops in the animals, so that their lower specific gravity must be due to the fact that their salt-content is less than that of sea-water. When placed in sea-water concentrated to half its volume they shrink, and when the sea-water is diluted with fresh-water they swell. The plasma membrane, therefore, shows the usual semipermeability to the balanced salts of sea-water—*i. e.*, impermeability to the salts and permeability to water. Out in the open bay, although most of the animals are at the surface on a calm night, many can be seen through a glass-bottom bucket well below the surface. Moreover, on windy days, they are not found at the surface, indicating that they have the power of increasing their specific gravity and sinking, owing to a temporary increase in permeability which allows the salts of sea-water to pass in or water to pass out. When noctilucas die they immediately sink, the permeability having been increased, causing an increase in specific gravity.

That these animals can also decrease their specific gravity is shown by the following facts. In all dilutions of sea-water with fresh-water down to 6 sea-water to 4 fresh-water, they rise immediately to the top, becoming and remaining somewhat swollen for more than 10 days. These dilutions are still of greater density than the animals. In a mixture of 6 sea-water to 4 fresh-water they remain distributed through the water for about 15 minutes, and then rise to the top; this mixture is of approximately the same density as the noctilucas. In a mixture of half sea-water and half fresh-water and also in a mixture of 4 sea-water to 6 fresh-water, the animals sink at first, their salt-content being now greater than that of the surrounding medium, but during the next hour they gradually swell and rise to the top. The process is wholly independent of movement of the tentacle. If water were merely absorbed by the animals until their concentration was the same as that of the surrounding medium, the animals would remain suspended through the liquid; but since they eventually float, they must keep on absorbing pure water until their salt-content and hence their specific gravity is again *less* than that of the new medium (5 sea-water to 5 fresh-water or 4 sea-water to 6 fresh-water), thus reestablishing their normal relation to their surrounding medium. This must involve the absorption of water against the osmotic pressure of the salts of sea-water, a condition contrary to physical laws. This regulatory mechanism is characteristic of living cells only and is not destroyed under any experimental conditions. Anesthetics, acids and alkalies, KCN, and pure salts of sea-water do not affect the regulation, except when they cause irreversible changes and death of the cells. It is quite probable that this peculiar type of osmo-regulation is not characteristic of noctilucas alone, but may be possessed by other marine plankton forms without gas-chambers which float and sink as occasion demands.

The water absorbed by noctilucas in mixtures of 5 sea-water to 5 fresh-water and 4 sea-water to 6 fresh-water accumulates in large vacuoles formed by strands of protoplasm pulling away from the cell-wall and forming membranes around the accumulated liquid. I have observed these vacuoles bursting or being expelled bodily from the cell. In these same concentrations of sea-water an interesting protoplasmic fragmentation also takes place, secondary smaller masses of protoplasm being formed, sometimes isolated and sometimes connected with the main mass by strands of protoplasm. The vacuoles formed by *Noctiluca* in these mixtures of fresh-water with salt-water are perhaps comparable with the contractile vacuoles of *Paramæcium*, *Amœba*, and other fresh-water forms, where its function is likewise osmoregulatory. It is true that in general in salt-water protozoa no contractile vacuoles are present, since the osmotic pressure of sea-water balances that of the animal and no osmoregulatory mechanism is needed. When *Noctiluca* is put under conditions where it must rid itself of a certain amount of fresh-water, similar though less perfect vacuoles can be formed.

In still more dilute mixtures of sea-water and fresh-water, the animals absorb water until they burst and then shrink; they are killed and sink to the bottom. That this is an osmotic effect and not due to the mere dilution of salts is shown by diluting the sea-water with isotonic cane-sugar, thus diminishing the salt-content but not the osmotic pressure. After testing various concentrations of cane-sugar to determine which was isotonic, it was found that the animals responded normally longest in m cane-sugar, so that this was used to dilute the sea-water. In m cane-sugar in distilled water the animals give a normal response for 2 hours; this is accompanied during the last hour by a steady glow; but when sea-water is added to the m sugar in various proportions, the effect on the luminescence is only slight (table 2). When 3 parts sea-water are added to 7 parts m sugar or any greater proportion of sea-water is used, the animals give a normal response for more than 9 days. In mixtures of 2 sea-water to 8 sugar a normal response is given for 4 days, and in 1 sea-water to 9 sugar a normal response is given for 2 days. The noctilucas, therefore, like muscle and other cells, must be bathed by a certain minimal salt-content as well as surrounded by a fluid with a certain osmotic pressure. They behave in this respect like the luminous bacteria investigated by E. N. Harvey (1914).

TABLE 2.—*Effect of m cane-sugar + sea-water.*

m cane-sugar to sea-water.	Luminous response.
m sugar........	Normal response 2 hours; constant glow during last hour.
9 to 1..........	Normal response 2 days.
8 to 2..........	Normal response 4 days.
7 to 3–1 to 9....	Normal response 9 days (experiment discontinued).

SALTS OF SEA-WATER.

In any one of the pure salts of sea-water, made up in m/2 solution in distilled water, *Noctiluca* ceases to give any luminous response after 2 hours (table 3). $CaCl_2$ is the most toxic, no normal response being given, but a constant glow for 2 minutes. KCl is least toxic, a normal response being given for 2 hours. In NaCl and in $MgCl_2$ there is a normal response for an hour, and this is accompanied on first immersion in the salt by a constant glow which soon passes off, doubtless owing to some of the animals dying. In a mixture of NaCl with one of the other salts, in the proportion occurring in sea-water, the normal response lasts much longer than in any of the pure salts; a little under a day in NaCl+KCl, and 5 days in $NaCl+CaCl_2$ or $NaCl+MgCl_2$.

TABLE 3.—*Effect of salts of sea-water.*

Solution (in distilled water).	Luminous response.
m/2 NaCl	Normal, 1 hour (poor); momentary constant glow at start.[1]
m/2 KCl	Normal 2 hours.
m/2 $CaCl_2$	Constant glow 2 minutes.
m/2 $MgCl_2$	Normal 1 hour; constant glow 10 minutes at start.[1]
m/2 (100 NaCl+2.2 KCl)	Normal, <1 day.
m/2 (100 NaCl+2 $CaCl_2$)	Normal, 5 days.
m/2 (100 NaCl+10 $MgCl_2$)	Normal, 5 days.
m/2 (100 NaCl+2.2 KCl+2 $CaCl_2$)	Normal, 5 days.
m/2 (100 NaCl+2.2 KCl+10 $MgCl_2$)	Normal, 7 days.
m/2 (100 NaCl+2.2 KCl+2 $CaCl_2$+10 $MgCl_2$)	Normal, 10 days.
Same mixture+m/2300 NaOH	Normal, 10 days.
Same mixture+m/2300 HCl	Constant glow 15 minutes.

In a mixture of $NaCl+KCl+CaCl_2$ a normal response is given for 5 days, and in $NaCl+KCl+MgCl_2$ for 7 days. With the four salts in a neutral or alkaline medium (+m/2300 NaOH), the response lasted for more than 10 days, when the experiment was discontinued. The addition of a small amount of acid (m/2300 HCl) to the four salts prohibits any normal response, causing a constant glow for 15 to 30 minutes. (See also under Acids.)

These results showing the injurious effects of pure salts on the luminous response of *Noctiluca* and the improvement on balancing with other salts are in agreement with the general work on the effect of balanced solutions on other physiological activities. The luminous bacteria, on the other hand, are independent of a balanced medium, although they must, like *Noctiluca*, have a certain small proportion of salt in the medium (E. N. Harvey, 1914).

---

[1] The constant glow at the start is doubtless due to the dying condition of some of the animals.

ACIDS AND ALKALIES.

For ascertaining the effects of acids on the luminescence of *Noctiluca*, hydrochloric (a lipoid insoluble) and benzoic (a lipoid soluble) acids were used, the solutions being made up in a neutral artificial sea-water, m/2(100 NaCl+2.2 KCl+2 $CaCl_2$+10 $MgCl_2$), so as to avoid the disturbing presence of the buffer phosphates and carbonates.

Certain concentrations of these acids, n/2000 to n/4000 HCl and n/4000 C_6H_5COOH, cause a bright continuous glow lasting from 20 to 60 minutes (table 4). The glow in these solutions becomes gradually brighter, stays at a maximum for some time, and then becomes gradually fainter. In n/4000 HCl the animals also give a normal response—that is, they flash on stimulation while they are giving a steady glow. Whether it is certain animals in the solution that give the one response at the same time that others are giving the other, or whether all of the animals give both responses, it was not possible to determine. Stronger concentrations of these acids kill the animals almost instantly, while weaker concentrations have practically no effect.

TABLE 4.—*Effect of acids and alkalies.*

Solution in artificial sea-water.	Luminous response.	Color change in neutral red.
n/500 to n/1000 HCl	Momentary constant glow.	
n/2000 HCl	Constant glow 30 min. (bright, becomes brighter, then fainter).	
n/4000 HCl	Constant glow and normal response 1 hr.	
n/8000 HCl	Normal 6 days (poor); momentary constant glow at start.[1]	
n/16000 HCl	>6 days (exp. discontinued).	
n/500 to n/2000 bensoic	Momentary constant glow.	
n/4000 bensoic	Constant glow 20 min. (becomes brighter, then fainter).	
n/8000 bensoic	>6 days (poor) (exp. discontinued); momentary constant glow at start.[1]	
n/16000 bensoic	>6 days (poor); (exp. discontinued).	
n/125 NaOH (in Mg-free sea-water)	Constant glow 2 min	4 min.
n/250 NaOH (in Mg-free sea-water)	Constant glow 2 min	10 min.
n/500 NaOH	Normal response 30 min., then constant glow 30 min.[2]	1 to 1½ hr.
n/1000 to n/2000 NaOH	Normal >7 days (exp. discontinued)	None.
n/250 NH_4OH	Constant glow 30 seconds	25 sec.
n/500 NH_4OH	Constant glow 75 seconds	30 sec.
n/1000 NH_4OH	Constant glow 4 min. (faint)	1 to 2 min.
n/2000 NH_4OH	Normal 3 min., then constant glow 6 min.[2]	None.
n/4000 NH_4OH	Normal 5 days	None.
n/8000 NH_4OH	Normal >7 days (exp. discontinued)	None.

The alkalies NaOH (lipoid insoluble) and NH_4OH (lipoid soluble) have an effect upon luminescence similar to that of the acids, except that the constant glow is more faint and of shorter duration; n/125 to n/250 NaOH and n/250 to n/1000 NH_4OH (the first two solutions were made up in Mg-free sea-water, all the others in artificial sea-water) cause a constant glow lasting from 30 seconds to 4 minutes, and no normal response is given (table 4). In slightly weaker solutions, n/500 NaOH and n/2000 NH_4OH, a normal response is given for a short period, owing doubtless to a delay in penetration, followed by a constant glow.

---

[1] The constant glow at the start is without doubt due to the dying condition of some of the animals.

[2] The normal response at the start is doubtless due to the delay in taking effect.

In one series of experiments comparison was made of the penetration time of the alkali and its effect on luminescence. The comparison was made on the same lot of animals stained with neutral red, which is not harmful to them. One set was observed in the dark for the effect on luminescence, while the other set was watched simultaneously by an assistant for color-change. In the case of NaOH the color-change from red to yellow takes place only *after* all light-response has stopped, whether this be a steady glow or a normal response; NaOH, therefore, does not penetrate the cell until after luminescence has ceased. On the other hand, with NH_4OH the color-change takes place *before* the effect on luminescence has stopped—that is, the alkali penetrates the cell while it is still giving a luminous response. For example, in n/500 NH_4OH the color-change takes place in 30 seconds, while the constant glow lasts 75 seconds. These results tally with those of E. N. Harvey (1914), who found that in certain plant and animal cells NaOH penetrates the cell only after its death, while NH_4OH penetrates before the death of the cell. The effect of alkalies on light-production is therefore brought in line with its effect on muscle-contraction, protoplasmic rotation, and other physiological processes.

POTASSIUM CYANIDE.

In strong KCN solutions, m/10 to m/250, a normal response is given at first, lasting from less than a minute to 30 minutes, and this is followed by a constant glow lasting for 7 minutes in m/10 and for 35 minutes in m/250 KCN (table 5). The normal response at the start is probably due to the delay in the KCN taking effect. In a concentration of m/500 the normal response lasts for several hours,

Table 5.—*Effect of potassium cyanide.*

Solution in sea-water.	Luminous response.[1]
m/10 (precipitate forms)	Normal response ¾ minute, then constant glow 7 minutes.
m/60	Normal response 4½ minutes, then constant glow 15 minutes.
m/125	Normal response 10 minutes, then constant glow 35 minutes.
m/250	Normal response 30 minutes, then constant glow 35 minutes.
m/500 to m/2000	Normal response >1½ hours, <12 hours.
m/4000 to m/16000	Normal response >6 days (experiment discontinued).

[1] The normal response at the start is doubtless due to the delay in taking effect.

and in solutions weaker than m/2000 there is no effect on luminescence. It is of interest to note the slight effect of KCN on oxidations connected with luminescence in comparison with its marked inhibiting effect on oxidations by plant oxidases and on the oxygen consumption of egg-cells and other animal tissues (Loeb and Wasteneys, 1913). The oxidations connected with light-production are apparently entirely different from those concerned with cell-respiration.

ABSENCE OF OXYGEN.

The effect of the lack of oxygen on *Noctiluca* was tested by subjecting the animals to an atmosphere of bubbling well-washed hydrogen from a Kipp generator. When thus treated, the luminescence gradually grows fainter for an hour until it is very faint. If air is now admitted to the tube, the animals respond on irritation as normally and just as brilliantly. Animals caught by the surface film of water on the sides of the tube give light when oxygen is admitted, even without mechanical stimulation. The mere admission of oxygen after its absence may therefore serve as a stimulus.

If the animals are kept for 2 hours, however, in the bubbling hydrogen, the luminescence goes out entirely and does not return on admitting air to the tube, the animals having probably been killed by the treatment. In a control experiment, hydrogen was bubbled through a tube containing the animals, in presence of oxygen; the animals were normal after 1 hour, but gave no response after 2 hours. It is therefore the mechanical disturbance and not the lack of oxygen which is fatal to the animals subjected for 2 hours to the hydrogen.

Light-production in *Noctiluca* is therefore dependent upon a supply of oxygen, as is to be expected. Since the cells deprived of oxygen immediately give light on admitting oxygen, even without stimulation, they must be permeable to oxygen at any time, and not merely upon stimulation and death, when light-production ordinarily takes place. That oxygen is necessary for light-production in *Noctiluca* is contrary to the ideas of Quatrefages (1850), and brings this animal in line with all the other light-producing animals in which the question has been carefully investigated.

TEMPERATURE.

With increase of temperature up to 42° or 43° C., noctilucas give a normal response. From this point to 48° or 49°, a steady glow is given and then the light goes out completely and there is no recovery if cooled immediately. With decrease in temperature, the animals flash more than normally until the temperature reaches 5° to 0°, when they give a constant glow. If kept only a few minutes at 0°, they will recover on warming and again give a normal response; but if kept at 0° for 15 minutes they do not recover.

CENTRIFUGING.

On centrifuging noctilucas for 3 to 5 minutes with a hand centrifuge, the animals form a layer at the surface of the liquid, and in each animal the protoplasm is thrown to the bottom of the cell as the heaviest material. The animals do not orient themselves in any way in the centrifuge, but the protoplasm is thrown to whatever part of the cell happens to be toward the centrifugal pole. After about an hour, many of the animals recover, and the protoplasm may be seen under the microscope returning to its normal position near the mouth-groove and flowing along protoplasmic strands to the periphery of the cell. No separation of the materials of the protoplasm into layers was observed with the centrifugal force used. The effect of centrifuging upon the luminescence is practically *nil*, for the animals give a normal response as soon as taken from the centrifuge tubes and placed in a dish of sea-water.

ELECTRICITY.

When a constant current is passed through a mass of noctilucas, the animals flash brightly at the make, continue glowing during the passage of the current, and cease to glow at the break, giving no flash, but sometimes they stay glowing after the break, and in this case the stronger the current the longer the glow lasts. If stimulated mechanically while the current is passing, they respond by a flash, just as when no current is passing.

The light comes from all parts of the noctiluca and is not restricted to anode or cathode regions. No increase in luminosity could be observed on the cathode side nor decrease on the anode side of the animal comparable with the polar effects of the current on muscle.

The tentacle movement is also influenced by the passage of a constant current. At the make, the tentacle coils up rather tightly, like a watch-spring, and at the break it uncoils, the process being repeated for a number of makes and breaks. This tentacle response is similar to the abnormal behavior of the sartorius muscle of a frog, which sometimes contracts on the make, stays contracted while the current is passing, and relaxes on the break. Spaeth (1916) similarly found that the pigment cells of fish-scales contract on the make, stay contracted during the passage, and relax on the break.

When a mass of noctilucas is subjected to the induced currents of an induction coil they respond with a flash at the break, and at the make also, if the current is strong enough.

If subjected to an interrupted induced current for 45 seconds, the animals flash on the first shock and then remain glowing, but the luminosity becomes gradually fainter. If the current is now stopped for a moment and then passed again, there is again a bright glow. The animals therefore fatigue rather readily when stimulated electrically, as they also do with mechanical stimulation.

RESPONSE OF INJURED CELLS.

If a number of noctilucas are punctured with a needle, causing the cells to collapse, and are then subjected to an interrupted current, they respond just as uninjured cells do. Such punctured and collapsed cells likewise give a normal response when stimulated mechanically—*i. e.*, by pressure of a cover-slip.

Noctilucas are injured by a few shocks of the strong induced current; the protoplasm shrinks away from the cell-wall, leaving a clear area at the periphery. If the animal is further subjected to an induced current, there is again a response, the protoplasmic area becoming luminous, while the clear peripheral area does not. After a short time the cell collapses and the animal appears as a shrunken mass of protoplasm within an irregular membrane. These shrunken animals also respond at the break of a weak induced current with a flash, just as normal cells do; and they likewise give a normal response to mechanical stimulation—*i. e.*, if pressed by a needle. It is thus shown that injury to a noctiluca does not interfere with its response to mechanical or electrical stimulation.

If, however, the injury to the cells is too great, and the cells are completely broken to pieces, they do not respond to stimulation. By pressing a mass of noctilucas through cheese-cloth, a filtrate was obtained containing many empty membranes and fragments of cells, visible under the microscope. This filtrate, although luminous, did not respond to electrical stimulation. Another filtrate obtained by pressing a mass of noctilucas through fine-meshed bolting-cloth, and tested while still luminous, did not respond to electrical stimulation. It is possible, however, that the fact that the noctiluca juice is acid may have some effect on the response in these cases.

LOCATION OF LUMINOUS MATERIAL.

When a noctiluca is giving a bright, constant glow—for instance, when treated with n/2000 to n/4000 HCl—it is fairly easy to observe the light under a microscope in a dark room. The chief luminescence comes from the main mass of protoplasm near the mouth-groove and around the periphery of the cell, while the area between these two regions is much fainter. The luminescence is a general glow over the cell, and a similar glow is suffused momentarily over the cell when a noctiluca is stimulated by a needle. This effect is, however, probably due to a close aggregation of small luminous particles, for when noctilucas are crushed under the microscope and the particles separated by means of a cover-slip, numerous points of light may be observed, similar to stars in the sky. The luminescence, therefore, apparently comes from small granules in the protoplasm, which may be freed from the cell by crushing. That the luminous granules are located in the protoplasm and not elsewhere was shown by stimulating cells injured by electricity. As mentioned previously, a few shocks of an induced current causes the protoplasm of a noctiluca to shrink away from the membrane into a compact irregular mass; when again stimulated, the irregular mass of protoplasm gives light, while the clear peripheral area remains entirely dark. Also, in centrifuged noctilucas there is a gradual motion of luminescence in a cell corresponding roughly with the motion of the protoplasm as it resumes its normal position. These granules in the protoplasm to which the luminescence may be traced do not stain with neutral red nor with methylene blue; there are other granules, however, especially around the periphery of the cell, which do stain with neutral red.

It has not been possible to obtain two substances concerned with light-production in *Noctiluca*, as in *Pholas* (Dubois, 1913, 1914), firefly and *Cypridina* (E. N. Harvey, 1917), etc., one destroyed by heat (photogenin) and the other thermostable (photophelein), notwithstanding repeated efforts to demonstrate them. Only one substance responsible for light-production in *Noctiluca* can be demonstrated, and this substance occurs as granules and burns until it is all used up as soon as it is brought into contact with atmospheric oxygen by crushing the cells; this we may conveniently call photogenin.

ANESTHETICS.

Anesthesia may be defined as a temporary inhibition of some physiological activity and includes a wide range of processes such as cell-division, responses to stimuli of various kinds, light-production, etc. Its chief characteristic is that the condition is reversible—that is, that normal processes are resumed on removal of the anesthetic. The anesthesia may be caused by various means—the constant electric current, change of temperature, and many chemical substances. Among the latter are the salts of Mg, Ca, and other metals, as well as the more common lipoid-soluble anesthetics—chloroform, ether, alcohol, etc. This study deals with the anesthesia of light-production in *Noctiluca* by means of the lipoid-soluble anesthetics.

The only previous work showing the definite anesthesia of light-production in animals is that of E. N. Harvey (1915) on luminous bacteria; he found that the light could be inhibited completely by various alcohols and returned on dilution with sea-water.

Of the various theories of anesthesia, that of Overton (1901) and Meyer (1899) has received widest acceptance. According to this theory, anesthesia is due to lipoid solubility, the anesthetic dissolving in the lipoids of the cell. It has been shown by Overton that there is a direct ratio between the narcotizing power of anesthetics and their lipoid solubility—that is, the more soluble the narcotic in fat, the more narcotic power it has and the smaller the amount necessary for anesthetizing. Just how the solution of the anesthetic in lipoids should change the irritability is not explained by the theory, nor does it take into account other anesthetics which are not lipoid soluble—*e. g.*, neutral salts. No distinction is made between the action of anesthetics in the membrane and in the cell interior. Traube (1913 and 1915) considers anesthesia as due to adsorption of the narcotic by surfaces in the cell, especially colloidal particles, due to the lowering of the surface tension of water. This causes a decrease in catalytic activity of the surface layer, leading to a decrease in the chemical activity of the cell, especially of oxidations. It has been shown that many substances with the same surface tension have the same physiological action, although this is not always true. The fact that dried micro-organisms free of lipoids can be anesthetized supports this theory rather than the lipoid theory (Warburg and Wiesel, 1912). On the other hand, the effect of temperature on anesthesia corresponds more closely with the effect on lipoid solubility than on adsorption of the anesthetic (Meyer, 1901).

That the narcotic acts by interfering with oxidation is held by others besides Traube. Verworn (1913) considers narcosis as an asphyxiation, since narcotized cells behave similarly to those deprived of oxygen. Mathews (1914) explains the relation of oxidation and anesthesia by assuming that protoplasm contains an unstable compound with oxygen, which breaks down on stimulation, and the anesthetic forms a stable compound with oxygen and thus prevents its liberation on stimulation. According to Mansfeld (1909), the anesthetic prevents the oxygen from entering the cell by decreasing the permeability of the surface for oxygen. In favor of these theories is the fact that oxygen consumption by certain cells and in certain oxidations has been shown to be decreased by anesthetics (Warburg, 1911). However, in these cases the concentration necessary to decrease oxidations is much greater than that necessary to produce anesthesia. Moreover, the experiments of Loeb and Wasteneys (1913) on sea-urchin eggs have shown that an anesthetic in the concentration sufficient to prevent cleavage had very little effect on oxidations. A slight decrease in temperature has a much greater effect on the rate of oxidations than an anesthetic of effective strength.

The membrane theory of Lillie (1909–1916) and Höber (1907) is also important. According to this the anesthetic affects the interior of the cell indirectly, the primary effect being upon the permeability of the cell membrane. The change in permeability is supposed to be connected with a change in the aggregation of colloidal particles, protein and lipoid. Lillie's experiments on *Arenicola* larvæ show that the narcotic prevents an increase in permeability, or in some cases causes an actual decrease. Osterhout (1913) has shown also, in *Laminaria*, that anesthetics cause a decrease in permeability, as indicated by an increase in resistance to the electric current. This theory takes into consideration the non-lipoid-soluble as well as the lipoid-soluble anesthetics.

All of these theories fall into two groups: those which consider the action of the anesthetic to be upon the cell-membrane and those which consider the action to be directly upon the cell-contents. Experiments on the anesthesia of light-production in *Noctiluca* have been carried out in the attempt to find out which of these two alternatives is correct.

Although the anesthesia of some processes has been shown to be independent of the consumption of oxygen—for instance, the cleavage of sea-urchin eggs (Loeb and Wasteneys, 1913, and Warburg, 1910)—it would seem probable that the anesthesia of light-production is dependent on oxygen. That oxygen is necessary for light-production in *Noctiluca* has been shown by keeping the cells in an oxygen-free atmosphere, in which case the luminescence stops, but reappears on admitting oxygen. The membrane is freely permeable to oxygen at any time, since those cells deprived of oxygen immediately glow on admission of oxygen. It may be noted in passing that this oxidation is different from that of other cells, as shown by the fact that KCN in relatively high concentrations has no effect, whereas it so quickly affects other oxidations.

The anesthesia must in some way affect the oxidation which is concerned in light-production. Does it do so by preventing oxygen from entering the cell, or does it prevent the utilization of the oxygen already present in the cell? In other words, does the anesthetic act upon the membrane or directly upon the cell interior?

In my experiments various lipoid-soluble anesthetics were tried and the effect of the anesthetic has been in all cases not to prevent light-production altogether, but to prevent a normal response—*i. e.*, a flashing on stimulation. In all the effective concentrations, the animals under the anesthetic produce a steady glow, so faint in some cases that it is not noticeable unless the animals are present in large number. When returned to sea-water, if not left too long in the solution, the steady glow ceases and the normal response returns; this is therefore a reversible phenomenon and a true case of anesthesia. The best concentrations for anesthetizing, as seen by referring to table 6, were: 1/3 saturated chloroform, where the steady glow lasted 2 hours; m/8 ether and m/8 butyl alcohol, steady glow lasting 1½ hours; 1/16 saturated thymol, lasting 1 hour; methyl alcohol, lasting 30 minutes, and 1/4 to 1/8 chloretone, lasting 15 minutes. If returned to sea-water after the period of steady glow, the animals gave no response, the prolonged anesthesia causing death. The tentacle motion was also stopped by the anesthetic in the same concentrations as prevented the normal light-response. The effect on the tentacle was, however, much slower than the effect on light-production, but took place during the early part of the period of constant glow.

The question as to whether the effect of the anesthetic is upon the interior of the cell or upon the cell membrane was tested by destroying membranes, thus allowing oxygen to enter. A number of noctilucas were subjected to m/8 butyl alcohol for an hour in a tube containing sand. Some were then returned to sea-water and recovered, showing that the animals were truly narcotised. The remaining animals were shaken up and broken to pieces by the sand. At the same time, a control lot kept for the same period in sea-water in a tube containing sand were shaken and broken up. A comparison of the two tubes showed that the anesthetized animals were not nearly so bright as the control lot. A microscopic examination of the material showed that the cells were completely broken to pieces into irregular fragments by the sand. A

TABLE 6.—*Effect of anesthetics.*

Solution in sea-water.	Luminous response.	Duration of tentacle movement.
Chloroform:		
Saturated	Constant glow 1 to 2 minutes.	
1/2 saturated	Constant glow 4 to 8 minutes.	
1/3 saturated	Constant glow 2 hours, then normal response, owing to evaporation of chloroform.	30 minutes.
1/4 to 1/8 saturated	Normal response >12 hours (exp. discontinued).	
Ether:		
m/2	Constant glow 4 minutes	<5 minutes.
m/4	Constant glow 30 minutes; becomes brighter, then fainter.	<5 minutes.
m/8	Constant glow 90 minutes	<30 minutes.
m/16	Normal response >1 day (exp. discontinued).	
m		
Ethyl alcohol:		
2m	Constant glow 1 minute	
1.5m	Constant glow 5 minutes	<5 minutes.
m	Constant glow 30 minutes	<10 minutes.
m/2 to m/4	Normal response >14 hours (exp. discontinued).	
Butyl alcohol:		
m/2		
m/4	Constant glow 1 minute	
m/8	Constant glow 5 minutes	
m/16 to m/32	Constant glow 1½ hours (faint)	<20 minutes.
	Normal response >14 hours (exp. discontinued).	
Chloretone:		
Saturated	No response	
1/2 saturated	Constant glow 4 minutes	Stops immediately
1/4 to 1/8 saturated	Constant glow 15 minutes (faint)	<10 minutes.
1/16 to 1/32 saturated.	Normal response >24 hours (exp. discontinued).	
Thymol:		
Saturated to 1/4 saturated.	Constant glow 1½ to 2 minutes	
1/8 saturated	Constant glow 6 minutes	
1/16 saturated	Constant glow <1 hour, normal response 4 minutes at start (poor).[1]	<15 minutes.
1/32 saturated	Normal response <18 hours (poor)	
1/64 to 1/128 saturated.	Normal response >5 days (exp. discontinued.	

[1] The normal response at the start is doubtless due to the delay in taking effect on certain of the animals.

similar experiment was tried with animals subjected to m/8 ether for 10 minutes with the same result. In these experiments the cell-membranes were destroyed, allowing oxygen to enter the cell directly, and the effect was different for the anesthetized cells and the control. The anesthetic must therefore act, not on the cell-membrane by preventing oxygen from entering the cell, but directly upon the cell interior, probably attacking the mechanism of the utilization of oxygen in the cell.

A criticism of the foregoing experiments might be offered—that, owing to the length of time that the cells were subjected to the anesthetic, substances present in the cells might be used up and the cells be fatigued. The following experiment showed that such was not the case: Three lots of animals were put into tubes with sand, two immersed in m/8 ether and the third in sea-water. One of the ether tubes was very slightly agitated almost immediately and did not increase in brightness as normal animals do on slight agitation, showing that the animals were narcotized. The other ether tube was shaken vigorously at once so as to break up the animals, and it became slightly brighter. The control tube with sea-water, when also shaken vigorously at the same time, became markedly brighter, showing a striking contrast to the other two tubes. In this experiment there was not sufficient time for any substances in the cells to be used up, and yet the narcotized cells gave a much weaker light than the control lot on admission of oxygen by destroying the membranes. The evidence from these experiments on *Noctiluca* would therefore argue against the membrane theory of anesthesia, since the anesthesia of light-production takes place independently of the cell-membrane; the effect of the anesthetic seems to be upon the mechanism of the oxidation process inside the cell.

SUMMARY.

1. The specific gravity of *Noctiluca* is less than that of sea-water, so that normally the animals float at the surface. Since they contain no air-bubbles or large oil-drops, their lower specific gravity must be due to a lower salt-content than that of sea-water. When placed in a mixture of 4 sea-water to 6 fresh-water, the animals sink, their salt-content being now greater than that of the surrounding medium, but later they rise to the surface, a process independent of the movement of the tentacle. They therefore absorb water until their salt-content is again less than the medium, thus reestablishing their normal relation to the surrounding medium. This water must be absorbed against the osmotic pressure of the salts of sea-water, a process contrary to physical laws. The animals can not only lessen their specific gravity, but can also increase it, as shown by the fact that they sink to the bottom of the sea on windy days. Anesthetics, acids and alkalies, KCN, and the pure salts of sea-water do not interfere with this regu-

latory mechanism, except when they cause irreversible changes and death; dead noctilucas always sink to the bottom.

2. Light-production in *Noctiluca* normally occurs only on stimulation of any kind, and is a momentary bright flash. The only other light-response is a steady glow occurring before death and under many experimental conditions—*e. g.*, diluting with fresh-water, addition of acids and alkalies, KCN, cold (5° to 0° C.), heat (43° to 49° C.), anesthetics, and a constant galvanic electric current.

3. In very dilute sea-water (3 sea-water to 7 fresh-water down to pure fresh-water) the animals give a constant glow for a few minutes and then die. If the sea-water is diluted with isotonic cane-sugar instead of water, a normal response is given even with 1 sea-water to 9 cane-sugar. But in isotonic cane-sugar alone a normal response is given for only a short period, followed by a steady glow and death, showing that the animals must be bathed by a medium of minimal salt-content as well as of definite osmotic pressure.

4. The normal light-response of *Noctiluca* is dependent on the balanced salts of sea-water. Isotonic Na, K, Ca, and Mg are all toxic if alone in solution.

5. n/2000 HCl and n/4000 benzoic acid in neutral artificial sea-water cause a steady glow for 20 minutes; n/2000 NH_4OH and n/500 NaOH give a steady glow for 6 and 30 minutes respectively. In NaOH the luminescence stops *before* the penetration of the alkali, and in NH_4OH *after* the penetration, as indicated by the color-change of animals stained in neutral red.

6. KCN has little effect on light-production in relatively strong concentrations. Animals in n/250 KCN respond normally for 30 minutes and then give a steady glow for 35 minutes.

7. Oxygen is necessary for light-production. Animals deprived of oxygen immediately glow on the admission of oxygen even without stimulation.

8. When the temperature is gradually raised, a steady glow appears at 42° C. and stops at 48°, when the animals die. When the temperature is lowered a steady glow appears at 5° and continues to 0°. The animals will recover on warming if kept for only a few minutes at 0°.

9. Centrifuging has no effect on the luminous response, although the protoplasm is thrown to whatever part of the cell happens to be away from the axis of the centrifuge. On standing, the protoplasm returns to its normal position under the tentacle.

10. With a constant galvanic current noctilucas flash at the make, stay glowing during the passage of the current, and stop glowing at the break with no flash. Animals will respond normally to mechanical stimulation during the passage of the current. The luminescence is uniform throughout the organisms, no polar effects being observed. Induced shocks call forth the usual flash caused by stimulation. The animals fatigue readily with interrupted induced shocks.

11. Noctilucas injured by puncturing with a needle or by a strong induced current respond to mechanical or electrical stimulation. If completely broken to pieces by pressing through cheese-cloth, the filtrate gives a constant glow, but there is no flashing on stimulation.

12. The luminescence is traceable to points of light coming from granules (photogenin) in the protoplasm. No photophelein could be demonstrated.

13. Noctilucas may be anesthetized by m/8 ether, 1/3 saturated chloroform, 1/16 saturated thymol, 1/8 saturated chloretone, m ethyl and m/8 butyl alcohol, so that they fail to give a flash on stimulation, but they always give a very faint glow; this disappears and the normal response returns on removing the anesthetic. Light-production is dependent on the consumption of oxygen. Whether the anesthesia of luminous cells is due to the fact that oxygen can not pass through the membrane, or to the fact that it can not be used, was tested by an experiment in which the cell-substance of anesthetized cells was permitted to come into contact with dissolved oxygen. Narcotized cells were broken up by shaking with sand and it was found that they produced only a faint light, whereas normal cells so treated became very brilliant. The anesthetic must therefore attack the mechanism of the utilization of oxygen in the cell, and not the permeability of the cell-membrane for oxygen.

LITERATURE LIST.

Dubois, R. 1913, 1914. Ann. de la Soc. Linn. de Lyons, vol. 60, 61.
Harvey, E. N. 1914. Carnegie Inst. Wash. Pub. No. 183, p. 131.
———. 1915. Biol. Bull. 29, p. 308.
———. 1917. Amer. Jour. Physiol., 42, p. 318.
Höber, R. 1907. Arch. f. die ges. Physiol., 120, p. 492.
Krukenberg, F. C. W. 1887. Vergl. physiol. Studien, Heidelberg, ii ser., pt. 4, p. 77.
Lillie, R. 1909 to 1916. Amer. Jour. Physiol., vol. 24 *et seq.*
———. 1916. Biol. Bull. 30, p. 311.
Loeb, J., and H. Wasteneys. 1913. Jour. Biol. Chem., 14, p. 517.
Mansfeld, G. 1909. Arch. f. die ges. Physiol., 129, p. 69.
Massart, J. 1893. Bull. Sc. de la France et de la Belgique, 25, p. 59.
Mathews, A. P. 1914. Intern. Zeit. f. Physik.-Chem. Biol., 1, p. 433.
Meyer, H. 1899. Arch. f. Exp. Path. u. Pharm., 42, p. 109.
———. 1901. *Id.*, 46, p. 338.
Osterhout, W. J. V. 1913. Science, 37, p. 111.
Overton, E. 1901. Studien über die Narcose.
Quatrefages, A. de. 1850. Ann. de Sc. Nat., iii ser., Zool., 14, pp. 226 and 236.
Spaeth, R. A. 1916. Amer. Jour. Physiol., 41, p. 577.
Traube, J. 1913. Arch. f. die ges. Physiol., 153, p. 276.
———. 1915. *Id.*, 160, p. 501.
Verworn, M. 1913. Die Narcose.
Warburg, O. 1910. Zeit. f. Physiol. Chem., 66, p. 305.
———. 1911. München. med. Wochenschrift, 58, p. 289.
——— and Wiesel. 1912. Arch. f. ges. Physiol., 144, p. 472.

X.

POLYCHÆTOUS ANNELIDS FROM FLORIDA, PORTO RICO, BERMUDA, AND THE BAHAMAS.

By AARON L. TREADWELL,
Professor of Zoology in Vassar College.

Three plates.

POLYCHÆTOUS ANNELIDS FROM FLORIDA, PORTO RICO, BERMUDA, AND THE BAHAMAS.

BY AARON L. TREADWELL.

INTRODUCTION.

In 1911 the present author published a paper entitled "Polychætous Annelids from the Dry Tortugas, Florida."[1] This was the first of a proposed series of systematic papers on the polychætous annelids of the West Indian region, collected under the auspices of the Department of Marine Biology of the Carnegie Institution of Washington, my main object having been the preparation of a monograph of the West Indian Leodicidæ. It has seemed best, since the final publication must be deferred, to publish a preliminary description of some new species belonging to this family, as well as new species of other families which have been collected incidentally in this work. I have included also a description of a new sabellid belonging to the collection of the American Museum of Natural History.

FAMILY SYLLIDÆ.

Odontosyllis octedentata n. sp.

A relatively large species, approximately 40 mm. long and 2.5 mm. in width. The prostomium (plate 1, fig. 1) is broader than long, with the anterior margin broader than the posterior, the lateral angles rounded. The posterior margin has a slight depression which is not shown in the figure because a lobe from the anterior margin of the peristomium extends over it. The palps are separate from one another, and prominent, not sharply separated from the prostomium, so that they seem almost to be forward prolongations of it. Eyes 2 pairs, brown, anterior pair the larger and farther apart than posterior pair. The median tentacle was noticeably more slender than the lateral and none of these extends much beyond the tip of the palp. None of the tentacles were well preserved and I am uncertain as to their normal appearance.

Pharynx short, with ventrally a row of 8 subequal teeth. The peristomium is prolonged dorsally into a broad lobe which covers the posterior surface of the prostomium. Tentacular cirri are slender, the dorsal ones being the largest. Dorsal cirri of later somites similar in form but successively smaller.

Parapodium (plate 1, fig. 2), with its end prolonged into equal anterior and posterior lobes and with an acicula with its apex coming to the surface just dorsal to these. Ventral cirrus ovate in outline, shorter than setigerous lobe. Dorsal cirrus long and slender. The setæ (fig. 3) with a very long basal joint, the longest reaching nearly halfway to the end of the dorsal cirrus. Terminal joint with a very short terminal and a single subapical tooth. Apex of basal portion with minute denticulations.

Type in American Museum of Natural History.

Collected at the Dry Tortugas in 1914.

---

[1] Bulletin of the American Museum of Natural History, vol. 30.

FAMILY POLYNOIDÆ.

Lepidonotus inquilinus n. sp.

Length of type 14 mm.; greatest breadth at end of anterior third 4 mm.; body tapering from here in both directions, but the head is broader than the pygidium.

The head (plate 1, fig. 4) is oval with the transverse diameter the longer, with a narrow median posterior depression and a much wider anterior one, the latter filled with the base of the median tentacle. Posterior eyes very small and situated on the dorso-posterior surface, the anterior ones larger, on the antero-ventral surface, and not visible from above. At the anterior margin the head is continued into the bases of the lateral tentacles, with a constriction at the base of the latter. Lateral tentacle not more than half as long as head, lanceolate, with acute tip. The other tentacles and tentacular cirri had been lost. Palps about 6 times the length of the head, perfectly smooth.

The body has 39 somites and 15 pairs of elytra. In alcohol the color is a yellowish brown with no trace of pigmentation except on the elytra. On each elytron a dark-brown pigment patch extends from the attachment of the elytrophore toward the median longitudinal line of the body. This is crossed by a definite white line at the position of the inner margin of the elytrophore and does not reach the edge of the elytron. (Plate 1, fig. 5.)

First parapodium (plate 1, fig. 6) with prominent antero-dorsal lip on the neuropodium, the notopodium slender and cirrus-like, with an acicula and a bundle of delicate, curved, minutely denticulate setæ. The ventral cirrus is very slender, reaching nearly to the tip of the neuropodium. A parapodium from the middle of the body (plate 1, fig. 7) has the neuropodia and notopodia much as in the anterior one, but the antero-dorsal lobe of the notopodium is more acutely conical in form. The dorsal cirrus has a stout basal joint, its terminal joint slender and tapering, extending beyond the apex of the parapodium. The ventral cirrus is situated on the neuropodium about one-third of its length from the apex. Each lobe of the parapodium has an aciculum and the arrangement of setæ is much as in the earlier somite.

In the middle parapodium are three kinds of setæ:

(1) Notopodial: these are all alike, each seta being very long and slender, curving gently toward apex and with very delicate teeth along one edge; fine lines across the seta possibly indicate minute plates of which the teeth are the free ends (plate 1, fig. 8).

(2) Dorsal neuropodial: with long slender shafts, but with the terminal third of the seta elongated lanceolate in form; minute transverse plates in this terminal third protrude from the margin so as to give it a minutely toothed appearance (plate 1, fig. 9).

(3) Ventral neuropodial: those lying next to the dorsal ones are much like them in length, but have heavier shafts and the terminal swelling is shorter, so that the lanceolate end is less elongated; the more ventrally placed ones are shorter, so that those on the very ventral side are not more than half as long as the dorsal ones; each (plate 1, fig. 10) has 5 or more rows of finely toothed plates beyond the base of the lanceolate portion, with very fine transverse plates between the last of these and the apex of the seta. The apex in both kinds of ventral setæ is bifurcate.

Collected July 6, 1915, on a *Marphysa* from Mangrove Key, in Key West Harbor.

Type in American Museum of Natural History.

Lepidasthenia varius n. sp.

Body variable in number of somites and length; one entire individual of 50 somites measured 30 mm., while an incomplete specimen contained 80 somites and measured 48 mm. Greatest width of larger specimen 3 mm.

The head (plate 1, fig. 11) with sides smoothly rounded laterally and tapering gently to the bases of the tentacles. The posterior margin is deeply incised and a faint groove is carried over the dorsal surface to the base of the median tentacle. The greatest width is behind the middle, and this is about equal to the length. There are apparently 2 pairs of eyes, the anterior larger and situated on the antero-lateral surface, the posterior near the posterior margin of the head. Neither eye showed any pigment, their position being indicated by an elevated patch of whitish epithelium.

The tentacles are carried on prominent basal joints, the median one being larger than either of the others. The median and one lateral were absent in both specimens. The remaining lateral was about 2½ times as long as the head, quite uniform in diameter except for a subterminal swelling and acute apex. Tentacular cirri similar in form to the tentacles, but larger and with traces of pigmentation near their ends. The palps are much longer than the cirri, tapering very gradually until near the end, then abruptly narrowing to an acute apex. With high power a few very small papillæ can be seen near the apex. The ventral cirrus of the second somite (first setigerous) and the dorsal cirri of the next somite much like the tentacles in form, but shorter and with less evident subterminal swellings. Later dorsal cirri are successively smaller and throughout the greater part of the body are conical, with acute tip. Of the ventral cirri, all but the first pair are small and conical; the anal cirri are much like the dorsal in form.

Elytra are carried on somites 2, 4, 5, etc., to 23, 27, and behind this two somites are regularly interposed between each pair of elytron-bearing somites. The first pair covers the head, while succeeding ones are smaller (*cf.* fig. 12, plate 1, of the first, with fig. 13, plate 1, of the eighth) and because of their small size and transparency they are easily overlooked and may seem to be absent. Toward the posterior end they are larger and cover more of the body, the terminal somites being entirely covered. They are all transparent, but behind the middle of the body each carries a patch of pigment, surrounding the point of attachment of the elytrophore and extending to the inner margin of the elytron. There is a very distinct line between this pigment and the point of attachment, giving the body in this region an ocellated appearance. The elytra all have smooth margins and are oval or round in outline.

The first parapodium (plate 1, fig. 12) cylindrical, very slightly expanded at apex, with presetal and postsetal lobes, the latter much the larger and curved upward at end. Between the two is a single row of stout setæ, their apices extending for only a short distance beyond the postsetal lobe. There is no indication of dorsal setæ, but two aciculæ extend, one into the dorsal part of the parapodium and one into the ventral.

The elytrophore is expanded at the apex, carrying the large rounded, nearly oval, elytron. The ventral cirrus is on a prominent cirrophore and is nearly 3 times as long as the parapodium, abruptly narrowing toward the end to form a slender acute tip.

The second parapodium is very similar to the first in appearance and arrangement of setæ (plate 1, fig. 14), with a dorsal cirrus resembling the ventral cirrus of the first parapodium. The ventral cirrus has a terminal joint in the form of a narrow cone, about as long as the parapodium. The fifteenth parapodium (plate 1, fig. 13) with very small achætous notopodium and a neuropodium much like those of earlier somites, but with a very small elytron. The ventral cirrus of the fifteenth is similar to that of the second. There is a single row of very stout setæ and there are aciculæ in both noto- and neuropodia. This general form of parapodium is continued to the end of the body, though the anterior and posterior lips of the parapodia become more nearly equal, so that the organ when viewed from above has a bifurcated appearance. The nephridial papillæ are cylindrical and very prominent.

The setæ are essentially alike throughout the body, differing only in the relative size of the different parts. Each has a stout shaft (plate 1, figs. 15 and 16), widening toward the end but narrowing to a pointed apex and with a sharp subterminal tooth. On their dorsally directed surfaces each has a series of alternately arranged plates with their free ends finely toothed. The setæ of the dorsal part of the setæ bundle have more numerous plates than ventrally, the middle ones being intermediate as regards this character.

One of the two specimens showed an indication of a brown color over the head and bases of the antennæ. Somites 2 and 3 were uncolored. From somite 5 to somite 20 the dorsum has a brown band, due to transverse markings in each somite; these are shorter than the somite diameter, so that the margins of the somites above the parapodia appear uncolored, but somites 6, 8, 12, 14, and 20 are uncolored, thus forming interruptions in this band. Behind somite 20 the markings occur only in the elytra-bearing somites.

Collected in the Dry Tortugas in 1914.

Type and cotype in the American Museum of Natural History.

Harmothoe variegata n. sp.

The type specimen was collected at the Dry Tortugas in 1909. I also have specimens collected at St. Augustine, Florida, and loaned to me by Professor Verrill.

The type is 26 mm. long and 5 mm. wide in its widest part, near the middle; from here it tapers toward both ends, but more noticeably so posteriorly than anteriorly. There are 39 somites, and 15 pairs of elytra.

The most striking part of the coloration of the alcoholic specimens is formed by the marking of the elytra. The inner and posterior margin of each elytron is marked with a dark line, which in the inner posterior quadrant is continued as a pigment patch, of a slightly lighter shade than the margin, to reach the elytrophore attachment (plate 1, fig. 17). When the elytra are in place the general effect is that of a broad median dark band on each elytron, the lateral posterior quadrant of each lighter-colored, but surrounded by a narrow marginal band. The remainder of the dorsal surface is uncolored, except that at the beginning of the posterior quadrant prominent black spots appear on the base and apex of some parapodia, usually only on elytra-bearing somites. Approximately the last 10 somites have a median dorsal pigment patch and 6 are uncovered by elytra.

Ventrally the margins of the mouth are pigmented, the pigmentation continuing on either side as a single patch at the base of each parapodium, toward the posterior end extending so as to cover a large part of the ventral surface.

The head is only slightly rounded, a little longer than broad, the anterior notch very narrow (plate 1, fig. 18). Peaks of head very slightly marked off from the ceratophores of the lateral tentacles, the latter about one-quarter as long as the head and densely pigmented. The terminal joint of the tentacle is slender, its entire length not more than twice that of the ceratophore, its tip filiform. The basal half of this terminal joint is pigmented, the terminal half being also colored, but not so deeply as the basal; apex uncolored. The median tentacle had been lost, but its ceratophore is large, overlapping the lateral ones, pigmented at its end. Palps rather stout, about 3 times as long as the head, their middle third pigmented. On the type there remained one ventral tentacular cirrus, slender and extending to about the middle of the palp, with a median and terminal pigment patch; all other tentacular cirri were lost, as were most of the dorsal cirri. Those dorsal cirri remaining were slender, gently tapering to the apex, more or less pigmented for the terminal half, but with a subterminal white patch. The anal cirri are larger than the dorsal and entirely pigmented.

The elytra are very similar in character throughout the body, with an entire margin and without papillæ; they are transparent, so that although they overlap, the pigment of the under one shows through the upper and gives the impression of a continuous line.

The notopodium of the first parapodium is thicker than the neuropodium and extends about half as far from the body. It bears from the bottom of an oval terminal depression a tuft of heavy setæ, of which the ventralmost are especially large (plate 2, fig. 1). The acicula extends into a conical expansion ventral to this depression.

Neuropodium much wrinkled, its dorso-anterior angle prolonged into a conical process; it carries a single acicula and large setæ. The ventral cirrus is large, equal in size to the dorsal cirrus of the second parapodium; its elytron was broken from the specimen figured, only its ceratophore being represented.

The second parapodium has a less developed notopodium than the first, but the setæ in the two are very similar. Neuropodium more conical than in the first, with a finger-shaped process at end of antero-dorsal lobe. Ventral cirrus two-thirds as long as the neuropodium, the dorsal cirrus longer than the parapodium, tapering gradually to the apex.

Toward the posterior end (plate 2, fig. 2) of the fifteenth from the posterior end the ventral cirri become very small, while an anterior lobe appears in the notopodium. In other respects the parts of the parapodium have a relative form much as anteriorly, and the very large setæ continue in the notopodium.

The large setæ of the dorsal tuft vary much in size, but the ventral ones are largest. They have a striated axis, which terminates in a blunt point and show traces of rows of minute teeth along the shaft. The apices were invariably covered with a thick brownish incrustation, which made it impossible to determine their exact form. The setæ of the ventral bundle (plate 2, fig. 3) with long tapering shaft and a subterminal tooth at some distance below the apex. The expanded portion of the seta with numerous rows of very minute teeth, apparently extending entirely around it, but larger on the convex surface; these stop at the subterminal tooth.

Type in American Museum of Natural History. Cotype in the Yale University Museum.

Pontogenia maggiæ Augener.

Pontogenia maggiæ Augener, 1906, p. 103.

A single specimen, dredged July 19, 1915, south of Loggerhead Key, agrees closely with Augener's description, though if his figure of the head is accurately drawn his specimen was poorly preserved. While there are lateral lobes as he figures, they are not as deeply incised as is shown in his figure and they are carried on the dorso-lateral surface, so that the head is broadest at its junction with somite 1. The median tentacle is shorter than the dorsal cirrus and very delicate. Each eye-stalk carries dorsally a small eye, and antero-ventrally a much larger one, somewhat as in Ehlers's *Pontogenia sericoma* (Ehlers, 1887, plate 7, fig. 2), but unlike that species these are carried on prominent stalks. Brown papillæ very abundant over the entire ventral surface, but occurring also on the head and dorsal surfaces of the elytræ, form a noticeable feature of this species.

Each somite with one or two very heavy, long, glassy setæ, longer than diameter of body and lying across its dorsal surface. If I understand correctly Augener's description, these are distinct from his "starke dorsale borste," which is shorter than these. I do not find in the latter any minute tubercles and would suggest that perhaps what he saw were particles of foreign matter.

FAMILY PHYLLODOCIDÆ.

Phyllodoce tortugæ n. sp.

Body of type 120 mm. long, of more than 200 somites. Prostomium 1.5 mm. in diameter, rather longer than broad (plate 2, fig. 4). Anterior and lateral margins of prostomium rounded, but posterior half of lateral margin nearly straight. Posterior margin deeply incised, and a nuchal papilla projects into this incision. Four subequal tentacles having rounded ends, their length not more than one-fourth that of head. Eyes one pair, large, black.

The ceratophores of all tentacular cirri are prominent, those of the dorsal ones on somite 2 being the longest. The terminal joint of the first cirrus and the ventral one of the second pair are very similar in form and size, while the dorsal one on somite 2 is the longest, reaching to the ninth somite; cirrus of third somite intermediate in length between the other two.

The partially protuded proboscis bears on either side a dense arrangement of disk-like papillæ, a median dorsal and ventral region being free from them.

The greatest width of the body, about 3 mm., was reached at somite 18. The anal cirri were much distorted in the type. On a cotype which was regenerating the posterior end, there was a single anal cirrus similar in form to the tentacular cirri, but much smaller.

Notocirri of anterior region obovate and inconspicuous in preserved material, standing well out from surface of body; they are larger and more conspicuous toward the middle of the body, and decidedly overlap one another, though at no time do they cover any very large part of the surface.

The parapodia (plate 2, fig. 5, of the tenth) with a bifid anterior lip, the posterior lip shorter and rounded (shown in dotted line in figure). A single acicula extends through the middle of the parapodium, the latter bearing on the end a row of about 10 compound setæ. The notocirrus is carried on a very stout ceratophore. The neurocirrus is on the postero-ventral face of the parapodium, its lower margin broadly rounded, bending up to form an acute angle with the straight upper margin. Later parapodia are similar to this in every detail, with the possible exception of a slight increase in the size of the notocirrus.

Seta from tenth parapodium (plate 2, fig. 6) slender with the apex of the basal joint covered with spines on its convex surface; terminal joint long, slender, fine-pointed, with row of minute teeth along the concave surface. The setæ of later somites resemble these in form, but the basal joint becomes longer, causing the seta to protrude considerably beyond the parapodium.

Type and cotype in American Museum of Natural History.

The type was collected at Loggerhead Key in the Dry Tortugas in 1914. The cotype was obtained by dredging about 10 miles south of Loggerhead in 1915.

FAMILY LEODICIDÆ.

Lumbrinereis cingulata n. sp.

An unusually small representative of this genus, the largest individuals being not over 40 mm. long with a prostomial width of 1 mm. The type after preservation was 37 mm. long and had 98 somites.

The prostomium when extended is broadly rounded, its length a little greater than its breadth (plate 2, fig. 7). There are no eyes, but some individuals have two relatively rather large pigment patches near the anterior border. The head, both dorsally and ventrally, is thickly studded with minute tubercles, clearly seen only under rather high magnification. These appear dark by transmitted, white by reflected light.

The first two somites are about equal in size (with tubercles similar to those on the prostomium), and each marked dorsally by two prominent bands of pigment spots. On later somites the tubercles are present, but except on the last two somites are less numerous than anteriorly. Anteriorly the somites behind the second have a narrow transverse band of pigment spots with occasionally much fainter bands at the margins of the tuberculated areas; they disappear entirely after the thirtieth somite. Ten or more of the most posterior somites have prominent ventral pigment patches which fuse to form a line that is wider in the center of each somite than intersegmentally. The body is very soft and easily broken, and secretes large amounts of mucus when put in clean water. There are two pairs of stout, unequal anal cirri.

The parapodia are similar throughout the body. Each (plate 2, fig. 8) has an anterior and a posterior lobe, the latter much the larger. I was unable to find any aciculæ. Setæ of two kinds. In the anterior somites the most prominent are the winged variety (plate 2, fig. 9); each has two winged processes and its axis is drawn out in an acute point, often much longer than in the one figured. At the sixteenth parapod in two specimens I examined (I am not sure that this is a constant position) the winged setæ disappear and hooded forms (plate 2, fig. 10) take their place. In most cases it seemed as if only winged ones were present in anterior somites, but in the fifth parapodium of one specimen was one hooded form which barely protruded from the surface.

The maxillæ (plate 2, fig. 11) are dark in color. The base is roughly triangular, very dark brown, with lighter margins. Basal portion of forceps rather broad and long, extending fully half the length, terminal portion slender and curved. A dark-brown pigment covers the terminal portion and continues as a dark margin along the inner edge of the basal portion, the remainder of the forceps being much lighter in color. The large paired plates have each 5 large, dark-brown teeth, the remainder being colored like the lighter part of the forceps. Of the two pairs of distal paired plates, the first has 3 teeth, the second 2; each is continued laterally into a chitinous plate dotted with black. The left-hand plates in the figure have been turned over. The mandibles are very delicate and difficult to separate from the maxillæ; they are nearly transparent, the only definite pigment being a dark spot on each near its outer anterior angle. The terminal beveled plate is not divided in the middle line. (Plate 2, fig. 12)

Collected at the Tortugas, where only 3 individuals were found, living in the crevices of broken coral, which make up a coarse gravel on parts of the bottom about 12 miles south of Loggerhead Key. They were common in Bermuda, living in the crevices of the porous surface of coral rocks, just below low-water mark, and some were found in a sponge. The type is from Bermuda.

Type in the American Museum of Natural History.

Marphysa viridis n. sp.

The type specimen, preserved and much contracted, was 100 mm. long, 2.5 mm. broad at peristomium, and 5 mm. broad at point of greatest width, which was at middle of body. It had approximately 200 somites.

In life the head and first somite are of a decidedly greenish color; remainder of body colored only by the blood seen through body-wall and gills. The head is distinctly bilobed and capable of considerable changes of form (plate 2, fig. 13). The tentacles are slender, colorless, and extend to the third somite. In life the eyes are prominent, but become less so after preservation. In preserved material the body as far as the ninth somite is very iridescent, but behind this it is of a uniform grayish brown.

Peristomium about as long as head, a little wider on posterior than on anterior margin, and only faintly marked off from the second somite, which is about half as long as it. Succeeding somites practically uniform in width and length until they narrow at posterior end. Anal cirri inconspicuous, but one of the two pairs much longer than the other.

The gills begin in the type specimen on the right side on somite 25 with a double filament; on the left they begin with one filament on 24, becoming double on 25. In later somites the filaments increase rapidly in length and new ones appear irregularly, the largest number I could find on the type being 5. Toward the posterior end the number again diminishes, and the last gill was on the twenty-fourth somite from the pygidium. The branches of all gills are long and straight, but except in contracted individuals they do not meet across the dorsal surface of the body (plate 2, fig. 14).

First parapodium with dorsal and ventral cirri about equal in length and with a conical post-setal lobe. The setal lobe bears several aciculæ (plate 2, fig. 15). The tenth parapodium with a rounded dorso-lateral post-setal lobe, the setal portion with two lobes, one dorso-posterior, the other ventro-anterior. The dorsal cirrus has a constricted base, then broadens, and finally narrows to a rounded tip. The ventral cirrus has a broad base of attachment and is bluntly rounded at apex.

A parapodium from the middle of the body has rounded setal lobe, whose anterior and posterior lips are about equal in size (plate 2, fig. 14). The dorsal cirrus is small and does not extend much beyond the setal lobe. The ventral cirrus has a broad base, and a small cylindrical terminal portion. The gill filaments are about equal in length and arise from a broad base. There are two dorsal aciculæ having rounded ends, while a ventral acicula with bifid end emerges just dorsal to the ventral cirrus. Behind the gill-bearing somites the cirri become more prominent, the dorsal being the more slender of the two.

Simple setæ slender with swollen pointed apex having entire edges. Compound seta (plate 2, fig. 16) has prominent serrations on the end of the basal joint, the terminal joint long, gently curved, without teeth. Pectinate setæ of two kinds; in the anterior somites they have about 20 very minute teeth, the end ones being a trifle longer than the others (plate 2, fig. 17). Posteriorly are found asymmetrical pectinate setæ (plate 2, fig. 18) flattened more than the others and with only about 8 prominent teeth. In the middle somites both kinds may occur. The dorsal aciculæ are rounded at the end, and are darker in anterior than in posterior somites. The ventral aciculæ are lighter in color than these, and are bifid at the end. In the posterior somites there occur, what I could not find in the anterior, needle aciculæ extending into the dorsal cirrus.

Jaws very dark, the forceps large with respect to the base, curved through 20°. The proximal paired plates have 5 teeth on the left side and 6 on the right. Distal paired plates with 7 on the right and 4 on the left. The unpaired plate with 8 (plate 3, fig. 1) teeth. The mandibles had very long, slender, dark shafts, the beveled portion being small and its surface white, except for a black outer edge (plate 3, fig. 2).

Collected July 1915 at Mangrove Key, Key West Harbor; Boca Grande; and at Marquesas, in sandy mud within digging distance of low-water mark.

Type in American Museum of Natural History.

Marphysa nobilis n. sp.

A large species, one specimen of average size measuring, after preservation, 350 mm. in length, with a peristomial diameter of 4 mm. The three somites following the prostomium were of the same breadth as the prostomium, but there was a later gradual enlargement to 7 mm. at the seventeenth somite. Behind this there was a gradual narrowing to the posterior end, this narrowing being more evident from somite 17 to somite 30. These measurements were taken on preserved material in which the relative proportions may not have been exactly as in life.

The body of the living animal is a flesh color due to the contained blood, and anteriorly is markedly iridescent. The only pigmentation of the surface is in the form of green bands on the otherwise colorless tentacles and numerous yellowish spots scattered over the anterior surface of the body. The posterior part of the body during life is decidedly transparent. Preserved material is uniformly brownish gray. In life, the red gills give a decided tint to the middle portion of the body.

The prostomium (plate 3, fig. 3) is bilobed, though the depth of the median depression varies at different times during life. When fully expanded its anterior edge is nearly straight and it tapers on either side to a narrower base. The tentacles are longer than the prostomium, approximately equal in size, and only very gradually tapering to the apex. The green color noticeable in living material is lost on preservation, and it is then seen that a constriction occurs at each green band; this resembles an articulation, but I think is not a true jointing. There is one pair of small eyes.

The peristomium is rather short, though its absolute size naturally varies with the degree of expansion. The constriction between it and the second somite is very obscure, so that the two are practically continuous.

The gills begin as a single filament on the region of the twenty-fourth to the twenty-ninth somite and acquire a second filament a few somites behind this. They later have as many as 6 filaments (plate 3, fig. 4) and when fully developed meet from opposite sides across the dorsal surface. Posteriorly they become smaller and finally disappear entirely at about 45 somites from the posterior end.

The first parapodium (plate 3, fig. 5) with prominent dorsal and ventral cirri and a long, cirrus-shaped, posterior lobe. The anterior lobe is truncated. Two short, very black aciculæ are in this parapodium and it carries dorsally a single tuft of needle setæ. The ventral seta tuft is short and lies behind the ventral cirrus and contains compound and pectinate setæ. The tenth parapodium has a large posterior lobe with the apex bent dorsally and a truncated anterior lobe. The ventral cirrus is conical, the dorsal with a decided lobe on its ventral surface. Three aciculæ extend beyond the end of the parapodium. Parapodium 150 (plate 3, fig. 4) shows much less distinction between anterior and posterior margins than do the anterior ones. The ventral cirrus is small, borne on a rounded base, the dorsal cirrus also small with a ventral lobe. The gill is rather thick and heavy at the base, but soon divides into filaments. The setæ are as in anterior somites and there are two aciculæ.

In the body at the base of the gill is a black spot. Toward the posterior end the form of the parapodium changes very little, but the cirri become slender and a ventral light-brown acicula makes its appearance.

The simple setæ are long, with a broadened terminal portion, but without noticeable fin or denticulations. The compound setæ have very long terminal joints, not toothed at the edge (plate 3, fig. 6). This terminal joint is relatively shorter through the gill region than anteriorly. Pectinate setæ of two forms; anteriorly with 20 to 25 relatively very short teeth, the terminal ones not very long; through the middle of the body a second form appears, having not over 12 very prominent teeth (plate 3, fig. 7). These are more numerous than the other form in the region of the gills. The dorsal aciculæ are black with rounded ends, the posterior ventral ones lighter in color and with a terminal bifurcation.

The jaw apparatus is dark brown. The basal portion of the forceps is short and the median portion rather long, terminal portion gently curved, concave on inner surface. In larger individuals each inner paired plate has 4 teeth, though in immature specimens the proximal tooth may not appear. Outer paired plates with 7 teeth on the right side, 4 on the left, 2 of these being much larger than the others (plate 3, fig. 8). Unpaired plate with 6 teeth. Beyond these teeth the margin is usually smooth, but denticulations may appear in it. On either side beyond the toothed plates is a thin plate with its upper angle curved to form a tooth-like process. The mandibles have rounded shafts and are lighter in color than the maxillæ, their apical beveled portion covered with a whitish deposit. (Plate 3, fig. 9.)

Collected first at Mangrove Key in Key West Harbor in sand exposed at extreme low tide, in June 1915. It was fairly abundant in this locality and more were collected there in July of the same year. A single specimen was collected in the same season at Long Key, at the Dry Tortugas.

Type in American Museum of Natural History.

FAMILY SABELLIDÆ.

Sabella alba n. sp.

Length 45 mm., of which the gills represent 10 mm. Body very slender, not more than 2 mm. in breadth at the collar.

Gills 17 pairs, colorless except for a fine longitudinal dusting of pigment along the bases of some of the rachises; bases of rachises connected by a very delicate membrane. On the outer surface of the rachises beyond the edge of the membrane are minute black ocelli, of varying number on different rachises and on different sides of the same rachis. These seem never to be more than 12 in a row. The extreme tip of each rachis is free from pinnules. The antennæ are slender, sharp-pointed, with (in alcoholic material at least) a noticeable white longitudinal median line. On either side, ventrally, is a patch of fine dark-brown spots similar to those found on the bases of some rachises.

The collar is rather low, its ends widely separated dorsally, but nearly in contact on the ventral surface (plate 3, figs. 10 and 11). The ventral ends have a fine recurved tip, but elsewhere the edge is straight and not recurved. On either side is a ventro-lateral fissure, giving the collar a 4-lobed character.

Body colorless except for a series of dark-brown ventral shields on the somites. Throughout the greater part of its length these shields are divided by a deep, narrow longitudinal fissure. There are 8 thoracic somites.

Thoracic setæ of two sorts, one (plate 3, fig. 12) lanceolate with asymmetrical expansion at the end, the other stouter, enlarged at the apex, shown in side view in plate 3, figure 13, and in full face in figure 14. The setæ of the collar fascicle are lanceolate like those shown in figure 12. Uncinus of thorax with a single tooth and a definite crest of minute denticulations, but without secondary teeth (plate 3, fig. 15). The pennoned setæ on the thorax have rather heavy stalks with very delicate, slender terminal portions. The tube is tough chitin, dark brown in color and very difficult to remove from alcoholic specimens without injuring the specimen.

Type in American Museum of Natural History.

Collected in the Dry Tortugas in 1914 and at Guanica Bay, Porto Rico, in 1915.

Parasabella sulfurea n. sp.

Length of body of type 65 mm., 18 mm. of which was in the length of gill.

The gills arise from a rounded basal portion as long as the first 4 body somites. There are about 15 on a side, of nearly uniform length, and with no inrolling on either edge. A very short portion of the apex of each rachis is free from pinnules. For a distance about equal to the length of the basal portion the rachises are united by a delicate web. Beginning just beyond the web and extending to beyond the middle of the rachis each carries on either side of its outer surface a row of minute purple spots. The basal portion of the gills is mostly of a purple color, while their greater part is sulphur-yellow, though an occasional pinnule is purple, but these are too few to affect the general coloration. Tentacles slender, pointed, about twice as long as basal portion of the gills.

A specimen collected in 1909 had, while living, a body bright yellow in color, with a greenish tinge dorsally and some purple markings on the dorsal surface of the collar. In alcoholic material the body is colorless, except for the ventral shields, which are a grayish brown throughout.

Collar rather low, its dorsal ends widely separated (plate 3, fig. 16) while ventrally the ends are nearly in contact (plate 3, fig. 17). Each ventral end is prolonged into a triangular recurved lobe, and at the base of the lobe is a pad-like thickening of the collar. The whole collar is very inconspicuous and has a recurved edge.

The torus of the first somite is arranged obliquely just posterior to the dorsal free end of the collar on either side (plate 3, fig. 16). On the next 7 somites the torus is lateral and ventral to the seta tuft. Beginning with the ninth somite and extending throughout the remainder of the body the torus is dorsal to the seta tuft.

Setæ of first setigerous somite of two sorts; one with a long, slender shaft (plate 3, fig. 18), the apex bent and narrowed to an acute point, with an indication of a lateral wing on both the concave and convex sides of the bend; the second form is stouter, with the apex rounded and covered with spines and terminating in a short, slender point (plate 3, fig. 19).

Setæ of later somites of four kinds. In the tuft about equal numbers of slender forms with curved apex, the bent portion covered with spines (plate 3, fig. 20) and of stouter forms with rounded ends (plate 3, fig. 21). In each torus is a single row of uncini, each with a single tooth and a long basal bar. Dorsal to the tooth are numerous fine surface striations, but no indication of secondary teeth (plate 3, fig. 22). In addition, below the uncini is a row of pennoned setæ (plate 3, fig. 23).

Common in the Tortugas, living in a dark-brown chitinous tube, usually in holes in solid coral rock. The tube is usually much longer than the animal and apparently its secretion keeps pace with the deposition of the rock.

Type in American Museum of Natural History.

I have included this in the genus *Parasabella* of Bush (1905, p. 191), distinguished from *Sabella* by the absence of a lateral incision in the collar lobe. In its general appearance and form of setæ it resembles *Protulides elegans* of Webster (1884, p. 325), but differs in the form of the collar.

Metalonome brunnea n. sp.

Length of largest specimen, including gills, 30 mm. Length of gills, 10 mm. Diameter of thorax, 3 mm.

There are about 13 gills on a side, with relatively long pinnules, which have a wavy outline, giving the whole gill a feathery appearance. The antennæ are short, broad at the base, with a very acute tip. The basal membrane of the gills extends for only a short distance up the plumule, and in alcoholic specimens this basal portion is a very dark brown, the color lightening very gradually toward the colorless apex.

Ends of collar widely separated on the dorsal surface, the dorso-lateral portions rather low, the edges not reflexed (plate 3, fig. 24). Ventral to the ventro-lateral notch the collar is produced on either side into a prominent pointed lobe (fig. 25), whose length is more than twice the width of collar. These lobes are in contact along the median line and cover a large part of the bases of the gills. Dorsal to them, between the bases of the gills, is a pair of lip-like folds, whose outer edges are in contact with and possibly are a prolongation of the ventral edges of the collar.

There are 12 thoracic seta-bearing somites. The first seta tuft is near the dorsal end of the collar, and there is no corresponding torus, while on the second and later seta-bearing somites there is a ventral torus. The torus becomes dorsal on the thirteenth setigerous somite. Ventral shields inconspicuous and divided longitudinally throughout the abdominal region by a narrow line.

The setæ of the collar fascicle are long, the terminal portion expanded laterally into a wing which is densely striated on its surface (plate 3, fig. 26, from a profile view). The thoracic setæ are in bundles, those of one side of each bundle being similar in form to the collar setæ, those on the other side having a terminal expansion ovoid when seen in face; between these two is a series graduating in form from one to the other; all have dense striations over the broadened surface. The uncini have a large tooth and a crest of 5 or 6 rows of smaller teeth (plate 3, fig. 27).

The tube is of thin white parchment covered at the end with fine white mud.

Type and cotypes in the American Museum of Natural History.

The specimens were collected at Nassau, British West Indies, by Whitheld.

I was unable to discover any pennoned setæ in the thorax of these forms, and consequently have included them in the genus *Metalonome* as defined by Bush (1905, p. 287).

LITERATURE.

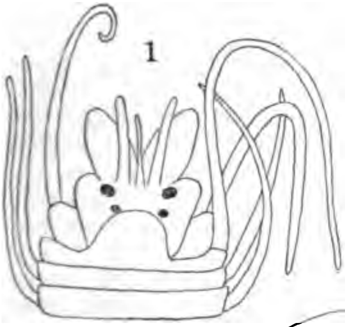
AUGENER, H. 1906. Westindische Polychæten. Bull. Mus. Comparative Zoology, Cambridge, vol. 43, No. 4.

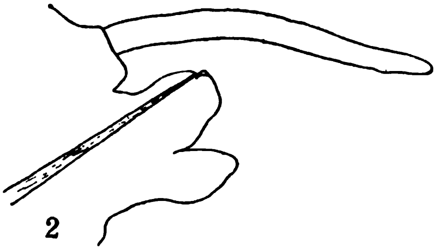
BUSH, KATHERINE J. 1904. Tubicolous Annelids of the Tribes Sabellides and Serpulides from the Pacific Ocean. Harriman Alaska Expedition, vol. 12.

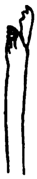
EHLERS, E. 1887. Florida Anneliden. Memoirs Mus. Comparative Zoology, Cambridge, vol. 15.

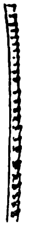
TREADWELL, A. L. 1911. Polychætous Annelids from the Dry Tortugas, Florida. Bull. Amer. Mus. Nat. Hist., vol. 30.

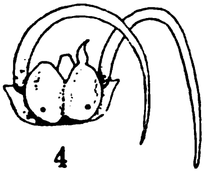
WEBSTER, H. E. 1884. Annelids from Bermuda collected by G. Brown Goode. Bull. U. S. Nat. Mus. No. 25.

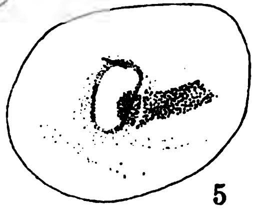

1

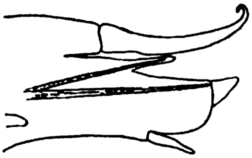

2

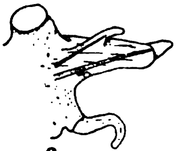

3

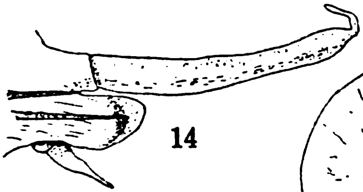

8

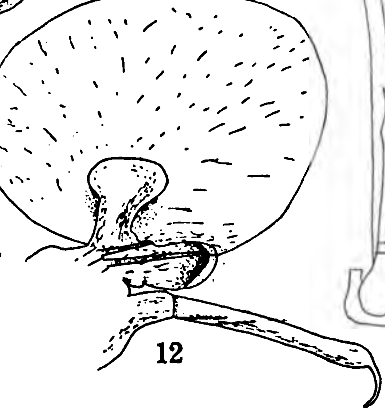

4

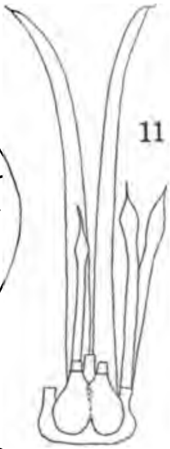

5

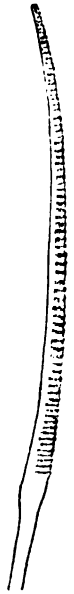

7

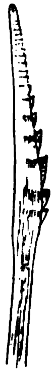

6

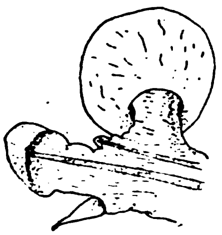

14

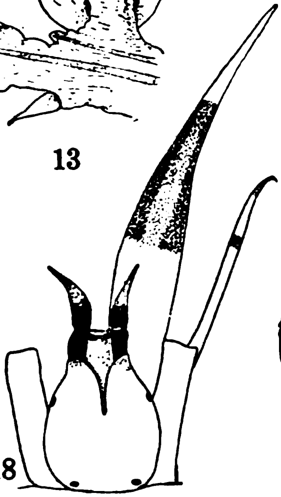

12

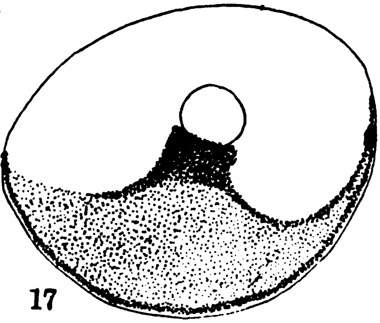

11

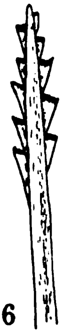

9

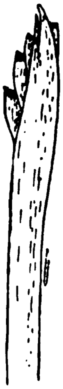

10


13


18


17


16


15

1 to 3. ***Odontosyllis octodentata*** **n. sp.:**
- **1. Anterior end, × 10.**
- **2. Parapodium, ×40.**
- **3. Seta, ×280.**

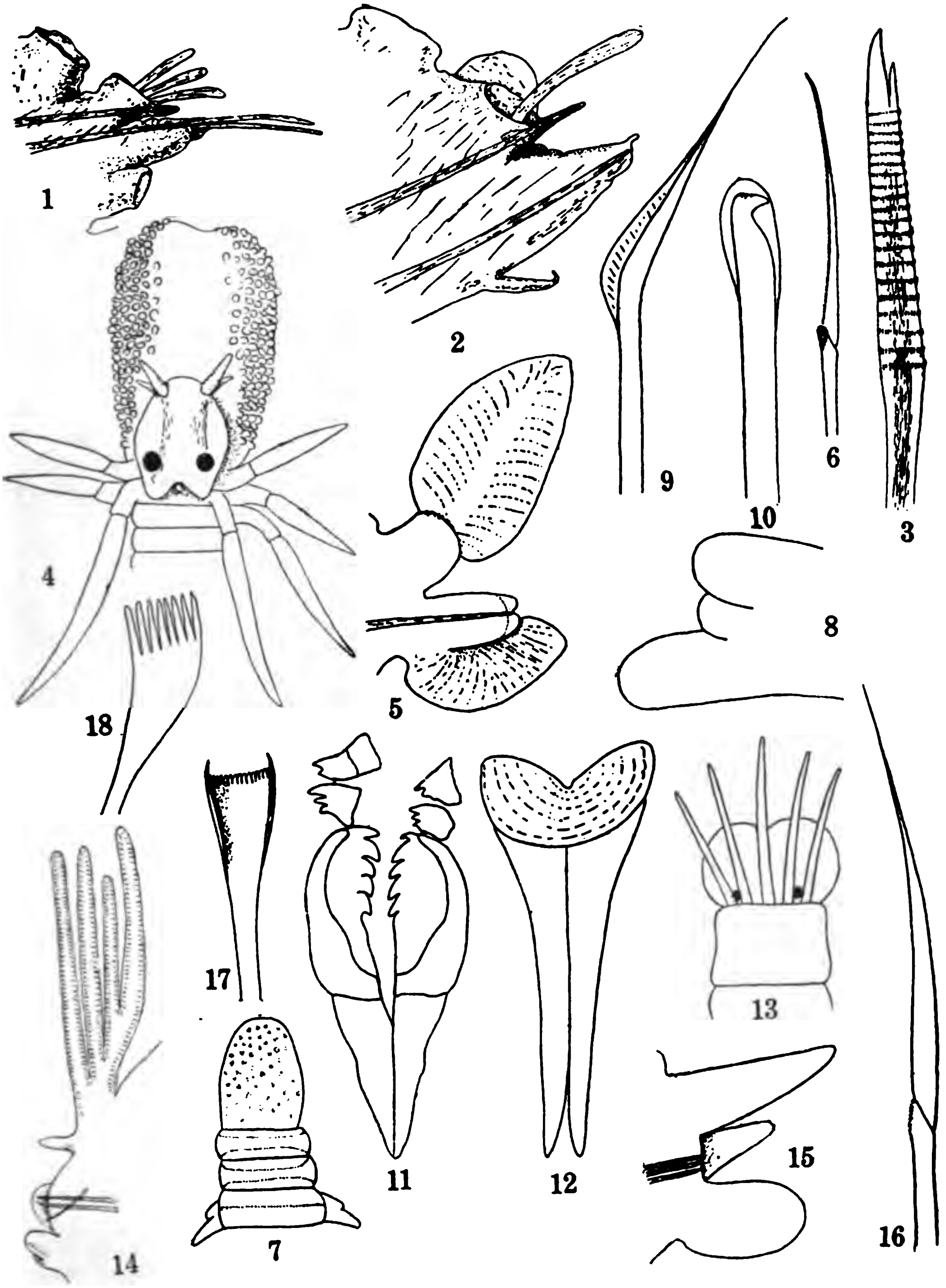
4 to 7. ***Lepidonotus inquilinus*** **n. sp.:**
- **4. Head, ×9.**
- **5. Elytron, ×25.**
- **6. First parapodium, ×28.**
- **7. Middle parapodium, ×28.**

8 to 10. ***L. inquilinus*****—cont.**
- **8. Portion of notopodial seta, ×280.**
- **9. Dorsal seta from neuropodial tuft, ×280.**
- **10. Ventral seta of neuropodial tuft, ×280.**

11 to 12. ***Lepidasthenia varius*** **n. sp.:**
- **11. Head, ×6.**
- **12. First parapodium, ×28.**

13 to 16. ***L. varius*****—cont.**
- **13. Eighth parapodium, ×28.**
- **14. Second parapodium, ×28.**
- **15. Lateral view of a seta from an anterior parapodium, ×280.**
- **16. Ventral view of the same seta shown in fig. 15.**

17, 18. ***Harmothoe variegata*** **n. sp.:**
- **17. Elytron, ×18.**
- **18. Head, ×8.**

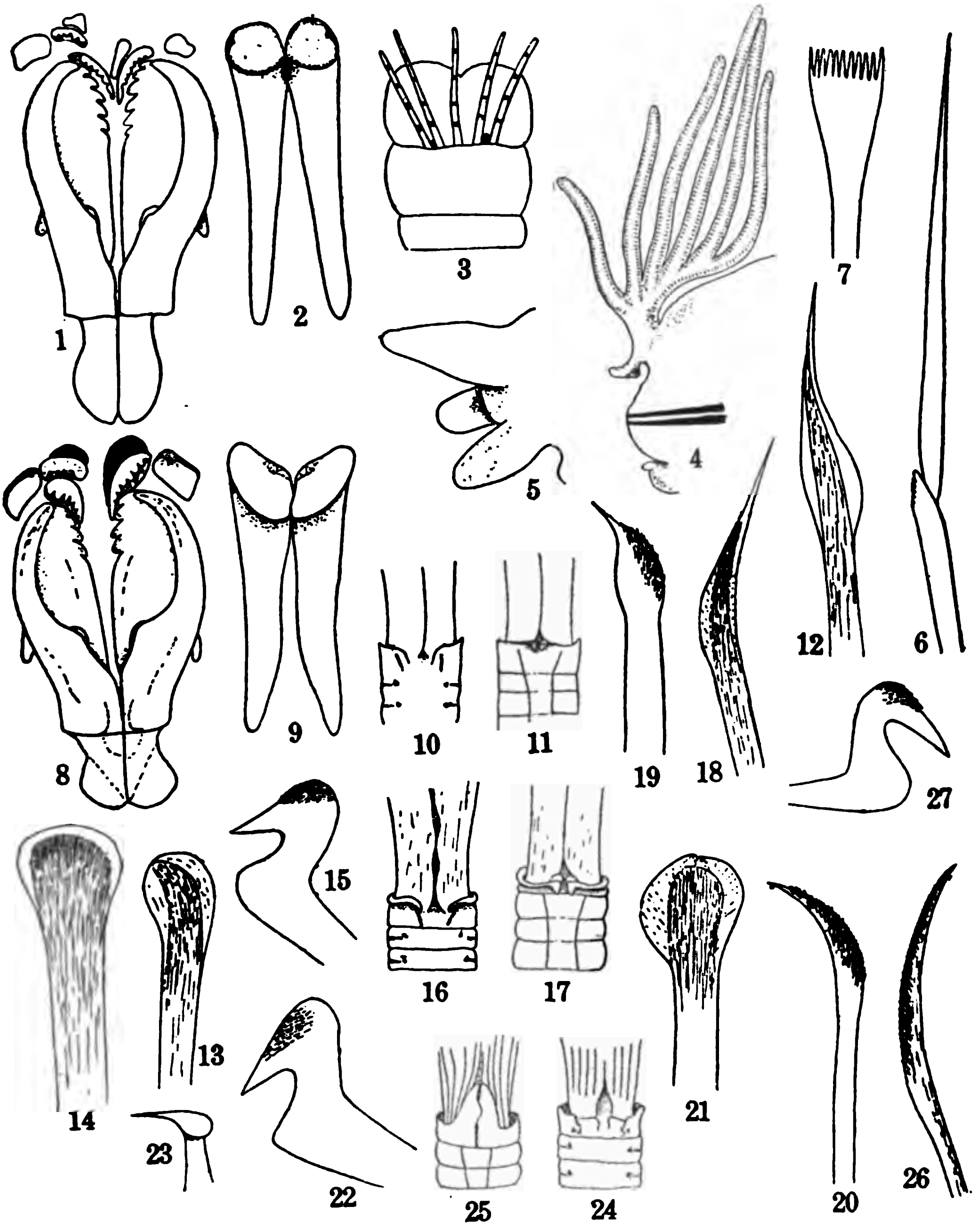


1 to 3. *Harmothoe variegata* n. sp.:
  1. First parapodium, ×28.
  2. Fifteenth parapodium from the posterior end of body, ×28.
  3. Seta from ventral tuft, ×280.

4 to 6. *Phyllodoce tortugae* n. sp.:
  4. Head, ×8.
  5. Parapodium from the tenth somite, ×45.
  6. Seta from the tenth parapodium, ×280.

7 to 12. *Lumbrinereis cingulata* n. sp.:
  7. Head, ×11.
  8. Parapodium, ×150.
  9. Winged seta from anterior somite showing on lateral view only one wing, ×230.
  10. Hooded seta from posterior somite, ×230.
  11. Maxillae, ×58.
  12. Mandible, ×58.

13 to 18. *Marphysa viridis* n. sp.:
  13. Head and anterior somites, ×7.
  14. Parapodium with gills from middle of body, ×38.
  15. First parapodium, ×38.
  16. Compound seta, ×370.
  17. Anterior pectinate seta, ×370.
  18. Posterior pectinate seta, ×370.



1 and 2. *Marphysa viridis* n. sp.:
- 1. Maxillæ, ×19.
- 2. Mandible, ×19.

3 to 9. *Marphysa nobilis* n. sp.:
- 3. Head, ×9.
- 4. 150th parapodium with gills, ×13.
- 5. First parapodium, ×27.
- 6. Compound seta, ×232.
- 7. Pectinate seta from middle of body, ×232.
- 8. Maxillæ, ×8.
- 9. Mandible, ×6.

10 and 11. *Sabella alba* n. sp.:
- 10. Collar and base of gills from ventral surface, ×5.
- 11. Collar and base of gills from dorsal surface, ×5.

12 to 15 *S. alba*—cont.
- 12. Lanceolate thoracic seta, ×280.
- 13. Club-shaped thoracic seta from side view, ×280.
- 14. Club-shaped thoracic seta from face view, ×280.
- 15. Thoracic uncinus, ×280.

16 to 19. *Parasabella sulfurea* n. sp.:
- 16. Base of gills and collar from dorsal surface, ×2.
- 17. Base of gills and collar from ventral surface, ×2.
- 18. Longer seta from first somite, ×280.
- 19. Shorter seta from first somite, ×280.

20 to 23 *P. sulfurea*—cont.
- 20. Slender seta from second somite, ×280.
- 21. Rounded seta from second somite, ×280.
- 22. Uncinus, ×280.
- 23. Pennoned seta accompanying the uncini, ×280.

24 to 27. *Metalonoma brunnea* n. sp.:
- 24. Collar and base of gill from dorsal surface, ×5.
- 25. Collar and base of gill from ventral surface, ×5.
- 26. Seta from collar fascicle, ×280.
- 27. Thoracic uncinus, ×280.

XI.

THE MICROSCOPIC STRUCTURE OF STRIPED MUSCLE OF LIMULUS.

By H. E. JORDAN,
Department of Anatomy, University of Virginia.

Three plates of sixteen figures.

THE MICROSCOPIC STRUCTURE OF STRIPED MUSCLE OF LIMULUS.

By H. E. Jordan.

INTRODUCTION.

The study of the skeletal muscle of *Limulus* was undertaken with two chief objects in view:

(1) To attempt further to test my conclusion suggested by a series of earlier studies on the intercalated disks of vertebrate cardiac muscle, namely, that these disks are properly interpreted as "irreversible contraction bands." If this conception is correct it seems probable that something very similar to intercalated disks may be found in powerfully acting skeletal muscle. Such structures have been repeatedly reported in vertebrate skeletal muscle, but the similarity to intercalated disks is still disputed. It must be emphasized, however, that even certain evidence of the presence of true intercalated disks in skeletal muscle is not crucial for the theory, but only confirmatory. Lack of such evidence can not invalidate the theory as applied to heart-muscle, nor does its possession necessarily prove it correct.

(2) To seek additional evidence in further refutation of the recently revived hypothesis that striped muscle can be interpreted in terms of "muscle-cells" and intercellular myofibrillæ.

A priori, the abdominal muscles which control the caudal spine of *Limulus* seemed to offer very favorable material for the search of the chief data desired. This surmise proved true beyond expectation as respects the second point. Moreover, the material yields very clear-cut data also as to a number of other disputed details concerning muscle structure, chiefly the relation of the ground membrane to the sarcolemma, to the nuclear wall, and to the myofibrillæ.

Concerning the first point no unequivocal evidence accrues. However, this study led to a reinvestigation of *Limulus* cardiac muscle with a different staining technic, and I can now report the presence of a very few, structurally of the simpler type, of intercalated disks. This observation would seem to warrant the generalization that cardiac muscle is characterized, at least as low as *Limulus*, by intercalated disks. The evidence concerning selachii and cyclostomes only remain uncertain. On the other hand, the evidence may perhaps be regarded as confirming the belief of some zoologists that *Limulus* is closely related to the more direct vertebrate ancestors. But similarity of histologic structure need not necessarily have phylogenetic significance; it may mean nothing more than the result of physiologic similarity. Obviously much comparative microscopic investigation of cardiac muscles is desirable below teleosts.

Other points of considerable interest touch the very close fundamental structural similarity between the spine-muscle and the heart-muscle of *Limulus*, and between the spine-muscle and an early stage of the development of striped muscle in teleosts.

MATERIAL AND METHODS.

As stated above, the material consists of spine and cardiac muscle. The former was collected during the summer of 1914, while engaged in the study of another problem at the Marine Biological Laboratory of the Carnegie Institution of Washington at the Dry Tortugas; the latter was collected at the Cold Spring Harbor Laboratory on Long Island Sound.

The cardiac muscle was fixed in Zimmermann's absolute alcohol-nitric acid solution, and stained with iron-hematoxylin and picric acid-fuchsin. The abdominal (spine) muscle was fixed both in Helly's fluid and in Flemming's strong solution, and also counterstained with the picric acid-fuchsin mixture.

SKELETAL MUSCLE.

GENERAL STRUCTURE.

The mass of muscle in the midline of the abdomen by which the spine (postanal telson) is moved consists of muscle-fibers varying greatly in diameter (fig. 1) and held together by a small amount of delicate wide-meshed connective tissue. The nuclei are scattered apparently promiscuously through the diameter of the fiber; as many as five (fig. 1), or even more, may be seen at approximately the same level in a cross-section of a large fiber. A narrow peripheral sarcoplasmic layer free of myofibrillar material envelops the muscle-fiber; occasionally nuclei occupy this location, even causing the confining sarcolemma to bulge slightly.

Under a magnification of 1,000 diameters, or even less, the fiber in cross-section is seen to have a radially striped appearance shading centrally into a mottled arrangement. Under higher magnification this appearance resolves into broad lamellæ, some apparently extending completely through the entire radius of the fiber. Many of the lamellæ appear split peripherally, producing a deep V- or Y-shaped structure. This represents a radial longitudinal splitting of the lamellar myofibril bundle. Centrally these lamellæ appear to split off smaller lamellæ and cylindric bundles of myofibrils. Between these lamellæ lies the delicate finely granular sarcoplasm (fig. 1B).

Of special interest is the similarity between this adult condition in *Limulus* and an early ontogenetic stage in striped muscle of teleosts.

The structure here described corresponds very closely to that described in the histogenesis of the striped muscle of the trout. It is desirable to know whether these muscle-lamellæ in *Limulus* also trace their origin to a single extranuclear fibril, as is the case in the cyprinoid fishes (Maurer, 1894) and in the trout (Heidenhain, 1913), a matter which is reserved for a future investigation.

Under closer examination these radial lamellæ and central straps and cylinders resolve into fibrils. The unit of structure, then, is the delicate myofibril variously associated into cylinders and lamellæ. These latter are apparently derived from the earlier larger lamellæ by process of splitting in two directions: (1) a radial direction, the splitting being initiated peripherally; (2) a vertical (paratangential) direction, which is chiefly confined to the central area of the fiber.

The limuli from which this tissue was derived were of medium size, *i. e.*, about half-grown. Probably the muscles enlarge by an increase in size of the smaller fibers by the process above described. In very thin longitudinal sections viewed under very high magnification this process of splitting appears to extend to the myofibrils themselves, the assumed units of structure. Indeed, this longitudinal splitting of myofibrils continues to the limit of visibility, and its variously consummated condition gives to the whole a syncytial structure even to its ultimate fibrils (fig. 2B). The impression becomes increasingly strong, as one improves the amplification and the acuity of observation, that the actual ultimate fibrils out of which the visible fibrillæ are formed are ultramicroscopic units—a confirmation thus far of Heidenhain's "Teilkörper theorie" ("histomere" or "protomere" theory), elaborated on the basis of his study of the development of trout skeletal muscle.

Viewed in longitudinal section, different fibers have a very different appearance, depending upon the functional phase of contraction or relaxation. In the uncontracted condition (fig. 2B) the *Q*-disk is conspicuous. Separating two successive *Q*-disks is an area of slightly greater width, very much less deeply stained, the *J*-disk; this is bisected by a deeper-staining but still relatively pale granular membrane, the telophragma (Krause's ground- or *Z*-membrane, 1869). Only the latter spans the intervals between adjacent fibrils; it is therefore a true membrane, as first recognized by MacCallum (1887) for heart-muscle, and as consistently urged by Heidenhain since 1899. The *Q* and *J* disks are regions of differentiation confined to the fibrils. Passing obliquely across the spaces, between adjacent myofibrils, are still more delicate fibrillæ, the ultimate visible units. At the point where the ground membrane and the myofibrils meet the latter appear to swell, giving to the membrane a deep-staining granular appearance; here the ultimate visible fibrils are apparently more closely associated into bundles, the so-called muscle-unit or sarcostyle. There is not the slightest indication in any fiber at any phase of function of any structure suggesting a mesophragma (membrane of Merkel, 1872; membrane of Heidenhain; *M*-membrane). Such a structure must be lacking in *Limulus* muscle; and in this respect it resembles the wing-muscles of certain insects, and the analogous muscles (pectoral muscles) of birds and bats as described by Thulin.

The fiber in the contracted condition shows only alternating darker and lighter stripes, the latter 2 to 3 times the width of the former. One can not properly speak here of *Q* and *J* disks. The fiber consists of a succession of contraction bands, composed of the ground membrane and *Q*-substance. The dark-staining stripes of contracted muscle correspond topographically to the telophragmata of the non-contracted fiber, but differ from it in that the fibril components are short rods instead of spherical granules.

THE TELOPHRAGMA.

The telophragma, as described above, is a continuous membrane, extending from sarcolemma peripherally to nuclear wall centrally, with both of which structures it is closely united, apparently continuous. Occasionally, where adjacent myofibrils are widely separated, a certain number of the *Z*-membranes can still be seen spanning the gap (fig. 3). The true-membrane condition of the telophragma is further proved by the appearance in certain fibers which have suffered distortion, apparently under pressure applied at right angles to the long axis (fig. 4). Such fibers exhibit a partial folding, the lines of folding (distortion) corresponding always with the telophragmata. The appearance is exactly such as would be expected to result if the fibrils were actually connected with each other by a membrane. The fiber could under the assumed condition, as it does, only fold in the same direction at any level along the lines of the membrane. Such fibers show beautifully also the granular swelling of the fibrils at the level of the membrane.

Centrally the membrane is closely related to the nuclear wall (fig. 3), peripherally to the sarcolemma (fig. 5). To the picric acid-fuchsin mixture the nuclear wall, *Z*-membrane, and sarcolemma have an identical staining reaction—that is, they either do not stain at all or, after long application of the dye, stain uniformly red.

THE SARCOLEMMA.

The muscle-fiber is enveloped by an extremely delicate sarcolemma (figs. 1B, 2A, 4, 5). There can be no doubt regarding its actual presence. Its demonstration is generally difficult in ordinary preparations, due to the very close investure of the endomysium, which is condensed immediately about the fiber. It is only after the study of differentially stained preparations that the distinction between sarcolemma and endomysium becomes marked; after that it can be identified as the innermost layer of the enveloping connective tissue, occasionally lifted away from both the fiber and the endomysium (fig. 4).

Its continuity with the *Z*-membranes marks it as cellular in origin, corresponding to the cell-membrane of the original myoblasts. In picric acid-fuchsin stained material the telophragmata and the sarcolemma remain unstained (appearing dark in such preparations), while the surrounding connective tissue (endomysium) stains a light red. When the staining process is unduly prolonged the sarcolemma also stains red, but then the *Z*-membrane and nuclear membrane and the peripheral sarcoplasm also take on the same red color.

THE NUCLEUS.

The nuclei lie scattered throughout the fiber, at any point on the radii, and are surrounded by a spindle-shaped mass of extremely attenuated sarcoplasm. These sarcoplasmic areas contain a small amount of granular material and several large lipoid spherules. This perinuclear sarcoplasm is bounded by myofibrils which converge apically. There is no suggestion of a cell-membrane. Indeed, that there can be no thought of cell structure is demonstrated by the relation of the telophragmata to the nuclear wall. These two structures are continuous; and the nuclear wall in many instances is drawn out into spines (ridges) at the point of connection with the membrane. In a contracted fiber such projections are less widely spaced, corresponding to the closer apposition of the membranes; in relaxed fibers they are spaced more widely and the projections are less sharp.

CARDIAC MUSCLE.

GENERAL STRUCTURE.

A cross-section of the tubular heart of *Limulus* shows the vast majority of the fibers cut longitudinally. They are collected into larger and smaller bundles, arranged approximately radially, tangentially, and circularly, crossing each other at angles of all degrees; a very few scattered cross-cut fibers may appear peripherally and centrally. The vast majority of the fibers are thus transversely disposed, only an insignificant moiety longitudinally. A median longitudinal section, on the contrary, shows a majority of the fibers cut transversely. Between these, and separating them into wider layers, are narrower bundles of longitudinally cut fibers, the radial fibers. One may search many sections cut near the medial longitudinal plane without seeing a single longitudinal fiber. The latter are certainly extremely rare, but a very few are apparently present, scattered peripherally and next the lumen. Both transverse and longitudinal sections immediately disclose an intricate loose-meshed muscular syncytium.

Figure 6A illustrates a cross-section under low magnification (1,000 diameters) of a large fiber at the level where it has become resolved into several main and a number of secondary branches. Each branch is enveloped by a delicate sarcolemma, the peripheral portion fusing intimately with the endomysium which incloses the entire bundle and separates the main branches. For purposes of minuter description a smaller trabecula, or branch, may be used. The myofibrillæ are grouped into large masses, in which they are arranged in lamellæ (fig. 6B) undergoing a peripheral radial and a central vertical splitting. The nuclei are located both centrally and peripherally.

Figure 7 illustrates the typical appearance of a small fiber. The myofibrillæ are segregated centrally and are enveloped by a considerable area of granular cytoplasm confined by a delicate sarcolemma. The nucleus here lies wholly in the extrafibrillar sarcoplasm. Upon close inspection the lamellæ are seen to consist of fibrillæ radially arranged. The apparent lamellæ illustrated in figure 6B are therefore smaller groups of fibrillæ.

As in the skeletal muscle, the cardiac fiber consists of peripheral lamellar and central cylindrical groups of fibrillæ. The close similarity between the cardiac and the skeletal muscle extends to the structure and distribution of the nuclei, the finer resolution of the apparent elementary fibrillæ (fig. 8), and the presence of a true cell-membrane or sarcolemma.

In longitudinal sections the appearance of the fiber again, as in the skeletal muscle, and almost identically, varies according to the degree of contraction. Certain fibers (fig. 9) show a wide darker-staining *Q*-disk and an alternating slightly wider *J*-disk bisected by a deep-staining granular *Z*-membrane. In certain other fibers, also uncontracted, only the deep-staining granular *Z*-membrane is conspicuous (fig. 11). The contracted fibers (fig 13c) show only a succession of contraction bands, alternating with lighter-staining disks. The contraction bands are very similar to those of the skeletal muscle and probably likewise represent a thickened *Z*-membrane, due to a segregation of *Q*-substance about them.

THE TELOPHRAGMA.

The telophragma is also a continuous membrane in cardiac muscle. It spans considerable intervals between widely separated adjacent fibrils (sarcostyles) (figs. 10 and 11); at its level the fibrils swell at the point of attachment; it is continuous peripherally with the sarcolemma (figs. 8, 10, 11, and 13) and centrally with the nuclear wall. That the granular appearance is actually due to swelling of the fibrils at the level of attachment is proved by the fact that that portion of the membrane which spans the space between the outermost fibrillæ and the sarcolemma, as also that which spans the perinuclear sarcoplasm, is non-granular (fig. 8). There is no suggestion of a mesophragma.

THE SARCOLEMMA.

That a true sarcolemma actually exists can not be doubted. This conclusion is contrary to that of Meek (1909). He holds that a sarcolemma is lacking, its place being taken by a connective-tissue envelope. But conditions are almost exactly like those described for the skeletal muscle. Successful counter staining with picric acid-fuchsin reveals internal to the red-stained endomysium an unstained, or only slightly stained, delicate layer with which the telophragmata are directly continuous. It is very intimately connected with the enveloping connective tissue (fig. 10), but occasionally it may be seen separated therefrom, as in figures 8, 11, and 13. When the staining is prolonged to the point where no distinction can be made on the basis of a difference in staining reaction between the sarcolemma and the endomysium, the telephragmata also, and even the peripheral sarcoplasm, show the same character and depth of stain. If we should decide on the basis of such a picture that the sarcolemma is in fact part of the connective tissue, we would be compelled to draw the same conclusion for the telophragmata and the peripheral sarcoplasm. Moreover, contrary to Meek's observation, the difference, both from the standpoint of size and structure, between the muscle nuclei and the connective-tissue nuclei, is striking, as can be seen from figure 13. The cytoplasm of the blood-cells and of the connective-tissue cells contains lipoid spherules similar to those of the perinuclear sarcoplasm.

THE NUCLEUS.

The nuclei are scattered apparently at random throughout the muscle trabeculæ. To their walls are attached the telophragmata, as in skeletal muscle. Hence, there can be no true cardiac-muscle cells. The cardiac muscle is an intricate syncytium, even to the finest visible fibrillar elements of the myofibrillæ. The most striking difference, and the only other essential difference, barring the gross syncytial character, is the generally much greater length of the nuclei; as many as 8 telophragmata ending on the periphery is a common condition (fig. 12); moreover, it is common to find 4 or more nuclei closely apposed in the same sarcoplasmic area. All the evidence goes to indicate extensive direct nuclear proliferation; all stages in amitosis can be readily demonstrated. Only rarely do 2 closely apposed nuclei in the same sarcoplasmic area appear in the skeletal muscle. This suggests, however, that the method of the nuclear multiplication is the same in the cardiac and skeletal type of muscle in the later growth stages—that is, by amitosis. Not a single karyokinetic figure was seen in the muscle-tissue of either type.

INTERCALATED DISKS.

Meek (1909) reported the absence of intercalated disks in the heart-muscle of *Limulus*. In this conclusion I confirmed him in an earlier study (1912). After reinvestigating the subject with a different staining technic (iron-hematoxylin and picric acid-fuchsin), I am able to report a very few disks of the very simplest or comb type (fig. 15). It was to be expected that intercalated disks should occur in a heart-muscle essentially so much like that of vertebrate cardiac muscle, with a similar rhythmic beat; and considering the low grade, and the slow rate of heart-beat (32 beats per second, Patten) of the organism, they were expected only in small number and of very simple form. These expectations are realized. However, the extreme rarity of the disks is surprising in view of the fact that they actually occur.

LITERATURE.

The only work known to me on the microscopic structure of *Limulus* muscle is that by Meek (1909) on the cardiac muscle. He describes its structure as a double syncytium composed of trabeculæ "individualized by connective-tissue sheaths"; the peripheral nuclei of the trabeculæ he interprets as *connective-tissue nuclei*. He records also the absence of sarcolemma and of intercalated disks, and notes the close similarity between the heart-musculature of *Limulus* and that of lower vertebrates. Carlson (1904) demonstrated the applicability of the neurogenic theory of heart-beat to *Limulus*. Meek concludes that a syncytial heart-musculature, accordingly, does not necessarily imply the verity of the myogenic theory of conduction. But this functional difference between *Limulus* and vertebrate hearts may in fact inhere fundamentally in the absence of an analogue of the vertebrate atrioventricular conducting bundle in *Limulus*.

The gross structural condition of the *Limulus* heart is very significant in this connection. The *Limulus* heart consists essentially of a metameric series of 9 syncytial muscular rings, for the physiologic coordination of which the very scattered peripheral and central longitudinal fibers seem quite inadequate. In the absence of a direct muscular coordinating mechanism the nervous impulse to heart-beat must be conducted by the longitudinal nerve-cord.

Patten (1912) only states that the striped muscle of *Limulus* arises very early from the somites. The histogenetic process is not described, but a detailed description is given of the origin and history of so-called "fiber cells," some of which give rise to definite muscles, others to "semi-amœboid cells resembling blood corpuscles." The original fiber-cells, derivatives of the germ-wall, are said to lie in the first 5 thoracic segments in an intermediate zone median to the germ-wall.

Other problems here considered are touched upon in the following recent works: (1) Baldwin's (1912) on the heart-muscle of the mouse, in which he describes "muscle cells" separated from the myofibrillar substance by a "cell membrane"; this interpretation was shown to be untenable in (2) my paper (1914) dealing with cat and mouse tissue in macerated condition, and by the findings of (3) Asai (1914) in his study of striped-muscle histogenesis in the mouse; (4) Thulin's (1915) work on the wing-muscles of certain insects (Coleoptera), birds, and bats, from which he records observations which he interprets as indicating the absence of both the meso- and the telo-phragmata, the so-called *Z*-stripe being the only striation discernible and to be interpreted as a contraction band; (5) Heidenhain's work on the histogenesis of striped muscle in the trout, on the basis of which he further supports and extends to striped-muscle tissue his general histologic principle of protomeric analysis, namely, the conception that muscle-tissue is built up by the association into successively larger combinations of ultimate fission elements, the metafibrillæ or protomeres; (6) Jordan and Steele's (1912) comparative study of the intercalated disks in vertebrate heart-muscle, from which it appears that intercalated disks are present in progressively simpler forms in all the vertebrate groups to and including teleost fishes; and (7) my description (1912) of the intercalated disks of hypertrophied human heart-muscle.

DISCUSSION.

The value of the data derived from the study of the *Limulus* muscle depends in degree upon the extent to which they may legitimately serve as a basis for generalization with respect to vertebrate muscle. In the relative simplicity of its striations the *Limulus* muscle seems more like that of vertebrates than like that of arthropods. Moreover, the fundamental close similarity between the cardiac and the skeletal type in *Limulus* is significant, especially as indicating that a main difference between cardiac and skeletal muscle generally is essentially a degree of differentiation, minute morphologic differences following functional differences probably largely inherent in the syncytial arrangement of the fibers in the heart-musculature. In both cases, as in vertebrate heart-muscle generally, the conspicuous stripe is the *Z*-membrane. Accordingly the skeletal muscle of *Limulus* is apparently less highly differentiated than vertebrate skeletal muscle, where the *Q*-disk gives the most conspicuous stripe, a conclusion further supported by the manner in which the nuclei are distributed. In its syncytial character and the presence of intercalated disks the *Limulus* cardiac muscle resembles very closely vertebrate cardiac muscle, a point already emphasized by Carlson and by Meek (8). The infrequency of the intercalated disks precludes an interpretation of these structures in terms of cell boundaries or intercellular substances, or as regions of growth (Heidenhain (12)). The disks consist of rows of modified foci in the fibrillæ. The modification consists of a change characterized tinctorially by an increase in staining intensity for a short distance on one or both sides of the telophragma. Structurally the disk is composed of a series of rod-like areas of the fibrillæ in transverse alinement. The most probable explanation of their formation is that of a change of position of the *Q* (anisotropic) substance from its usual location in relaxed fibers midway between two successive telophragmata, to a location on either side of these membranes. This explanation answers to the description of the formation of a contraction band. In that a disk appears permanent, it seems appropriately described as an "irreversible contraction band." In view of what was known of the comparative morphology of intercalated disks—coupled with our knowledge of the minute structure of *Limulus* heart-muscle—the simple comb type was to be expected in *Limulus*. This type of disk is actually present. But disks were expected in greater number. Possibly hearts of older limuli would show more abundant disks.

The precise information furnished by the *Limulus* muscle concerning the relation of the ground membrane to the myofibrillæ is of prime importance in respect to the interpretation of occasional serrated disks of apparently normal cardiac muscle and the still more complex disks of hypertrophied heart-muscle. This general type of disk is of very irregular serrated structure. The matter which was difficult of interpretation hitherto was the nature of the cross-connections, in the form of delicate membranes, between adjacent elements of the disk proper. In view of the data now available, namely, the close connection of the myofibrillæ to the telophragmata and the divisibility of the so-called myfibril units into still finer fibrillæ, the matter becomes clear. The essential condition in muscular hypertrophy is an increase in size of the fibers due fundamentally to a longitudinal splitting of the myofibrillæ. Keeping in mind the connection of the myofibrils with the membrane, and the unequal tensions (relative or absolute) under which the adjacent muscle-fibrils work in the hypertrophied heart, the series of changes from the simpler comb type of disks to the complex serrated type of hypertrophied muscle become intelligible (fig. 16).

Heart-muscle, then, is clearly a syncytial structure in vertebrates and *Limulus*, and intercalated disks are presumably invariably present (the matter has not yet received attention below selachii), representing contraction bands which have become incapable of reversion, and thus undergo structural and chemical changes.

We are now in a position also to bring into harmony the discrepant descriptions of the relation of the simpler intercalated disks to the telophragmata. Heidenhain (12) describes the disks as invariably bounded on both sides by a ground membrane. They have also been variously described by other observers as bordered only on one side by a telophragma; and as having no definite relationship to these membranes. My own observations on mammalian cardiac muscle led me to conclude that the disks are generally bisected by a telophragma; occasionally they appear bounded on one side by this membrane; and generally in favorable instances the disks can be seen to shade laterally into a *Z*-membrane. If an intercalated disk of the comb type is correctly interpreted in terms of a contraction band, as I have maintained, then it becomes a simple matter to explain the usual relationship of the *Z*-membrane to the disk, as also the several occasional variations when the membrane forms either one or both boundaries. The *Z*-membrane forms also the mid-portion of the contraction band. As an "irreversible contraction band," an intercalated disk also is therefore usually bisected by the *Z*-membrane. It may conceivably occasionally happen that only half of the contraction band may be able to relax, whereas the other half may become converted into an intercalated disk. Such a disk would then be bounded only on one side by a ground membrane. If these same conditions should prevail in two successive contraction bands, involving the adjacent halves of the bands, then the resulting disk would be bounded on either side by a *Z*-membrane. Such disks would be rare, the prevailing type being bisected, or bounded on only one side, by the membrane.

The presence of a sarcolemma distinct from the endomysium is proved by the difference in staining reaction of the more delicate inner and coarser outer layers of the closely enveloping connective tissue. The outer reacts to specific stains for connective tissue; the inner does not so react, at least not to the same degree, but always to the same degree to which react also the telophragmata with which this inner layer is in close structural connection. This inner layer is a true sarcolemma. That it represents the original cell-membrane seems proved by the fact that it is connected with the nuclear wall through the telophragmata. The close association of sarcolemma and endomysium must be emphasized; but this is exactly the same as obtains in the case of the skeletal muscle, and exactly the same structural conditions obtain. In neither case can there be any doubt about the presence of a sarcolemma. The fact that one is present in *Limulus* cardiac muscle should go far to remove further skepticism regarding its actual presence in vertebrate heart-muscle.

Neither in the cardiac nor the abdominal-spine muscle of *Limulus* is there any indication of a mesophragma. Thulin records the absence also of a mesophragma, and even of a telophragma, in the wing-muscles of certain insects, birds, and bats. The meaning of this structural peculiarity in these muscles is obscure. Heidenhain (12) claims that a mesophragma is present even in human heart-muscle. The occurrence of such a membrane in cardiac muscle is disputed by many histologists. When apparently lacking, Heidenhain believes that it has a thickness of less than 0.2 micron, the limit of microscopic resolution, hence indiscernible. It seems obvious that the question as to whether heart-muscle anywhere actually contains mesophragmata demands reinvestigation. If it were actually present and of the same nature as the telophragma, and if it had the same relation to the myofibrils and to the sarcolemma, as Heidenhain believes, then it should cause festoons in the sarcolemma like those caused by the telophragma, which is not the case. Furthermore, if present, it should produce a similar folding to that produced by the telophragmata in certain distorted fibers, as shown in figure 4, which also is not the case.

In this connection should be considered the careful work of Meigs (16) (1908) on the wing-muscle of the fly. He recognizes the existence of at most only 3 different substances in the sarcostyle, that of the *Q* bands and that of the *Z* and *M* lines. He regards *J* as the optical effect of reflection phenomena due to the presence of *Z*. He conceives of *Z* as a substance more highly refractive than its surrounding medium *Q*. His identification of *M* as a true membrane in the wing-muscle of the fly is at variance with the observations on the wing-muscle of beetles by Thulin (4) who records its absence in this group. In *Limulus* striped muscle, both cardiac and skeletal, *M*-membranes can not be discerned either in fresh or fixed and stained material.

In unstained skeletal fibers the *Q*-band is faintly visible; in cardiac muscle it can only occasionally be barely detected. But in stained fibers in certain instances it is distinctly visible in both types of muscle (figs. 6B and 9). Certainly *Q* and *J* have a different staining capacity; and this indicates a structural difference, perhaps inhering only in a relatively greater abundance of "anisotropic granules" in *Q*.

The *J*-band, compared with the *Z*-membrane, is much too wide to permit of any reasonable interpretation of its appearance in terms of refraction phenomena due to the presence of the *Z*-membrane. Moreover, in the contracted fiber one sees only "contraction bands" (apparently $Z + Q$), and *J*-bands, the *J*-band being now topographically in part at the former level of the original *Q*-band. In the case of *Limulus* striped muscle, one is forced to conclude that 3 distinct substances are present, namely, that of the telophragmata and the *J* and *Q* substances.

Limulus increases in size at least throughout early life. Histologically the tissues of the internal organs mature early, but provision must be made for constant enlargement. This fact must be kept in mind in the interpretation of the structure of its muscle. Though mature as concerns its fundamental histogenesis, it presents developmental phenomena. It is of cardinal interest and significance that these are very similar to early histogenetic stages in the muscle of higher forms. In skeletal muscle this point concerns chiefly the arrangement of the fibrillæ in lamellæ and cylinders, each undergoing longitudinal splitting, the former both radial and vertical (paratangential). This is exactly the condition prevalent in developing muscles of the newly-hatched rainbow trout and other teleosts. In cardiac muscle this same point concerns also the nucleus. These nuclei multiply greatly, and by amitotic division. The reason for amitotic multiplication rather than mitotic in the enlarging *Limulus* muscle, both skeletal and cardiac, remains obscure.

This leads naturally again to a consideration of the evidence for "muscle cells" in the sense of Apathy (1888) and Baldwin (1912).

The original myoblasts are lost in a syncytium, in the skeletal muscle appearing as multi-nucleated distinct fibers, in the cardiac muscle as a loose-meshed intricate network of larger and smaller trabeculæ. The muscle-substance here consists of a finely granular sarcoplasm (with large lipoid spherules in the vicinity of the nucleus), throughout which are scattered the myofibrils and the nuclei. The relationship of ground membrane to nuclear wall and to sarcolemma, as also the result of the abundant nuclear amitosis in cardiac muscle, would hardly be intelligible on any other ground. In view of the evidence derived from the study of the muscle from the adult mouse (Jordan), and the data recorded by Asai (3) on the histogenesis of this same heart-muscle, the cellular hypothesis of muscle structure must be definitely abandoned.

If additional evidence were required in refutation of the interpretation of interfibrillar muscle-cells in striped muscle, it may be drawn from the structure of the striped muscles of tunicates (*e. g.*, tail-muscles of *Amaroucium*); here the "fiber" is a mononucleated elongate hexagonal cell, with its nucleus centrally and the myofibrillæ peripherally disposed.

The observations here recorded with respect to the fibrillar structure of *Limulus* muscle, namely, the resolution of the muscular substance into finer and finer complexes to the limit of visibility, strongly support the protomere hypothesis of Heidenhain (5), which maintains that all living matter is divisible into progressively smaller specific units, the ultimate vital molecular units being the "histomeres," "protomeres," or "metafibrillæ."

SUMMARY.

1. Both the skeletal and the cardiac muscles of *Limulus* consist of trabeculæ of finely granular sarcoplasm, holding regularly aggregated collections of myofibrillæ, and confined by a cell-membrane or sarcolemma; throughout the trabeculæ are scattered irregularly the numerous nuclei.
2. In cardiac muscle the main trabeculæ and their branches form a loose-meshed syncytium.
3. Neither type of muscle contains mesophragmata.
4. Very rarely an intercalated disk of the simple-comb type appears in the cardiac muscle.
5. Both types are very similar in respect of the presence and arrangement, in the same phase of contraction, of *Q* and *J* disks, and the telophragmata.
6. The telophragmata are continuous membranes closely attached centrally to the nuclear wall, which is frequently drawn out into projections at the points of attachment, and peripherally to the sarcolemma.
7. The sarcolemma is a very delicate membrane, closely associated with the enveloping endomysium, but reacting differently to specific connective-tissue stains.

8. The evidence is unequivocal against an interpretation of structure in terms of "muscle-cells" and intercellular myofibrillæ.

9. The nuclei of the growing muscles multiply by amitotic division.

10. The myofibrillæ may be resolved into still finer fibrils to the limit of visibility, an observation in support of Heidenhain's "teilkörper" ("protomere") theory.

11. The structurally different constituents of the *Limulus* sarcostyle are the *Z*-membrane and the *Q* and *J* disks. *Q* and *J* most probably differ only in the matter of a relatively greater abundance of certain darker-staining materials, the so-called anisotropic granules, in the former.

12. *M*-membranes occur only in certain more specialized types of striped muscle; they are probably never present in the form of actual membranes in cardiac muscle.

13. In essential structure the cardiac and skeletal muscles of *Limulus* are closely similar, indicating a close functional similarity. The structure serves, moreover, as a splendid illustration of the "law of biogenesis," in that it is practically identical with a stage in the early histogenesis of striped muscle of teleosts.

BIBLIOGRAPHY.

1. **Baldwin**, W. M. 1912. Muscle-fibers and muscle-cells of the adult white mouse heart. Anat. Anz., Bd. 42, Nu. 7 and 8.
2. **Jordan**, H. E. 1914. The microscopic structure of mammalian cardiac muscle with special reference to so-called muscle cells. Anat. Rec., vol. 8, No. 9.
3. **Asai**, T. 1914. Beiträge zur Histologie und Histogenese der quergestreiften Muskulatur der Säugetiere. Archiv mikr. Anat., Bd. 86, Nu. 1 and 2.
4. **Thulin**, I. 1915. Ist die Grundmembran eine konstant vorkommende Bildung in den quergestreiften Muskelfasern? Archiv mikr. Anat., Bd. 86, Nu. 3 and 4.
5. **Heidenhain**, M. 1913. Ueber die Entstehung der quergestreiften Muskelsubstanz bei der Forelle. Beiträge zur Teilkörpertheorie, II. Archiv mikr. Anat., Bd. 83, Nu. 4.
6. **Jordan**, H. E., and K. B. **Steele**. 1912. A comparative microscopic study of the intercalated disks of vertebrate heart muscle. Am. Jour. Anat., vol. 13, No. 2.
7. **Jordan**, H. E. 1912. The intercalated disks of hypertrophied heart muscle. Anat. Rec., vol. 6, No. 9.
8. **Meek**, W. 1909. Structure of *Limulus* heart muscle. Jour. Morph., vol. 20, No. 3.
9. **Jordan**, H. E. 1912. The intercalated disks of atrophied heart-muscle. Proc. Soc. Exp. Biol. and Med., vol. 10, No. 2.
10. **Baldwin**, W. M. 1912. The relation of muscle-cell to muscle-fiber in voluntary striped muscle. Zeits. f. Allgem. Physiologie, Bd. 14.
11. **Patten**, W. 1912. The evolution of the vertebrates and their kin. Blakiston.
12. **Heidenhain**, M. 1911. Plasma und Zelle.
13. **MacCallum**, J. B. 1897. On the histology and histogenesis of heart-muscle cell. Anat. Anz., Bd. 13.
14. **Maurer**, F. 1894. Die Elemente der Rumpfmuskulatur bei Cyclostomen und höheren Wirbeltieren. Morph. Jahrb., Bd. 21.
15. **Carlson**, A. J. 1904. The nervous origin of the heart-beat in *Limulus* and the nervous nature of coördination or conduction in the heart. Am. Jour. Phys., vol. 12, p. 67.
16. **Meigs**, E. B. 1908. The structure of the elements of cross-striated muscle, and the changes of form which it undergoes during contraction. Zeits. f. Allgem. Physiologie., Bd. 8, Nu. 1.

DESCRIPTION OF ILLUSTRATIONS.

Fig. 1A.—Transverse section of 5 adjacent skeletal-muscle fibers (abdominal-spine muscles), showing the great variation in diameter and the random distribution of the nuclei (*n*). ×750. Flemming's fixation; iron-hematoxylin stain.

Fig. 1B.—More highly magnified portion (*x*) of the largest fiber shown in A. *n*, nucleus; *s*, sarcolemma; *p*, perimysium (endomysium). The myofibrils are arranged in bundles throughout the sarcoplasm and show a peripheral radial and a central vertical (paratangential) division. ×2,000.

Fig. 2.—Longitudinal sections of: (A) Contracted fiber; (B) uncontracted fiber. *Z*, telophragma; *Q*, anisotropic disk; *J*, isotropic disk. ×2,000.

Fig. 3.—Portions of longitudinal sections of muscle-fibers showing a smaller (*A*) and larger (*B*) nucleus. The perinuclear sarcoplasm contains fine lighter and coarse darker (*g*) spherical lipoid granules. The telophragma (*Z*) is continuous with the nuclear wall, which projects into a series of spines at the points of union. ×2,000.

Fig. 4.—Striped-muscle fiber which has suffered distortion. The lines of distortion are identical with the telophragmata (*Z*), demonstrating the attachment of the myofibrils to the ground membrane. The myofibrils stain more intensely and are slightly swollen, giving a granular appearance at this level, at the point of attachment to the telophragma. *S*, sarcolemma. ×2,000.

Fig. 5.—Portion of longitudinal section of skeletal-muscle fiber showing the telophragmata (*T*) continuous with the sarcolemma. *E*, endomysium; *M*, myofibril; *S*, sarcolemma. ×2,000.

Fig. 6A.—Transverse section of a large trabecula of cardiac muscle, showing the division into several larger and a number of smaller branches. The trabecula and branches are enveloped by a sarcolemma (*S*) in intimate association with the endomysium (*E*). The nuclei are scattered promiscuously throughout the diameter of the fiber. ×1,000.

Fig. 6B.—More highly magnified portion (*x*) of A, showing arrangement of myofibrillæ into lamellar and cylindrical groupings. ×2,000.

Fig. 7.—Transverse section of small cardiac-muscle trabecula, showing the disposition of the larger bundles (*M*) of myofibrils (in which the fibrillæ are grouped in lamellæ and cylinders), the enveloping finely granular, delicate sacroplasm, and the confining delicate sarcolemma (*S*). The nucleus in this section is peripherally placed. ×2,000.

Fig. 8.—Portion of longitudinal section of cardiac-muscle trabecula. The telophragmata are continuous with the sarcolemma (*S*) which is festooned between the membranes. That portion of the membrane to which the myofibrils are attached is granular in appearance; the extra fibrillar portion is non-granular. The sarcoplasm is finely granular. The endomysium (*E*) is very intimately attached to the sarcolemma, but with picric acid-fuchsin counterstain it stains red, while the sarcolemma remains unstained. When the action of the stain is prolonged, endomysium, telophragma, sarcoplasm, and sarcolemma all take on a red or pink color. ×2,000. Zimmermann's fixation; iron-hematoxylin, with picric acid-fuchsin counterstain.

Fig. 9.—Fiber in the uncontracted condition, showing the *Z*, *Q*, and *J* lines. ×2,000.

Fig. 10.—Fiber showing the relationship of the telophragmata and the endomysium (*E*) to the sarcolemma (*S*). The endomysium is red in color, while the sarcolemma and telophragmata remain dark brown or black, in successfully acid-fuchsin stained preparations. ×2,000.

Fig. 11.—Cardiac fiber in the uncontracted condition, showing the deeper-staining granular modification of the myofibrillæ at the levels of attachment to the telophragmata. A peripheral nucleus and the sarcolemma (*S*) are also shown. ×2,000.

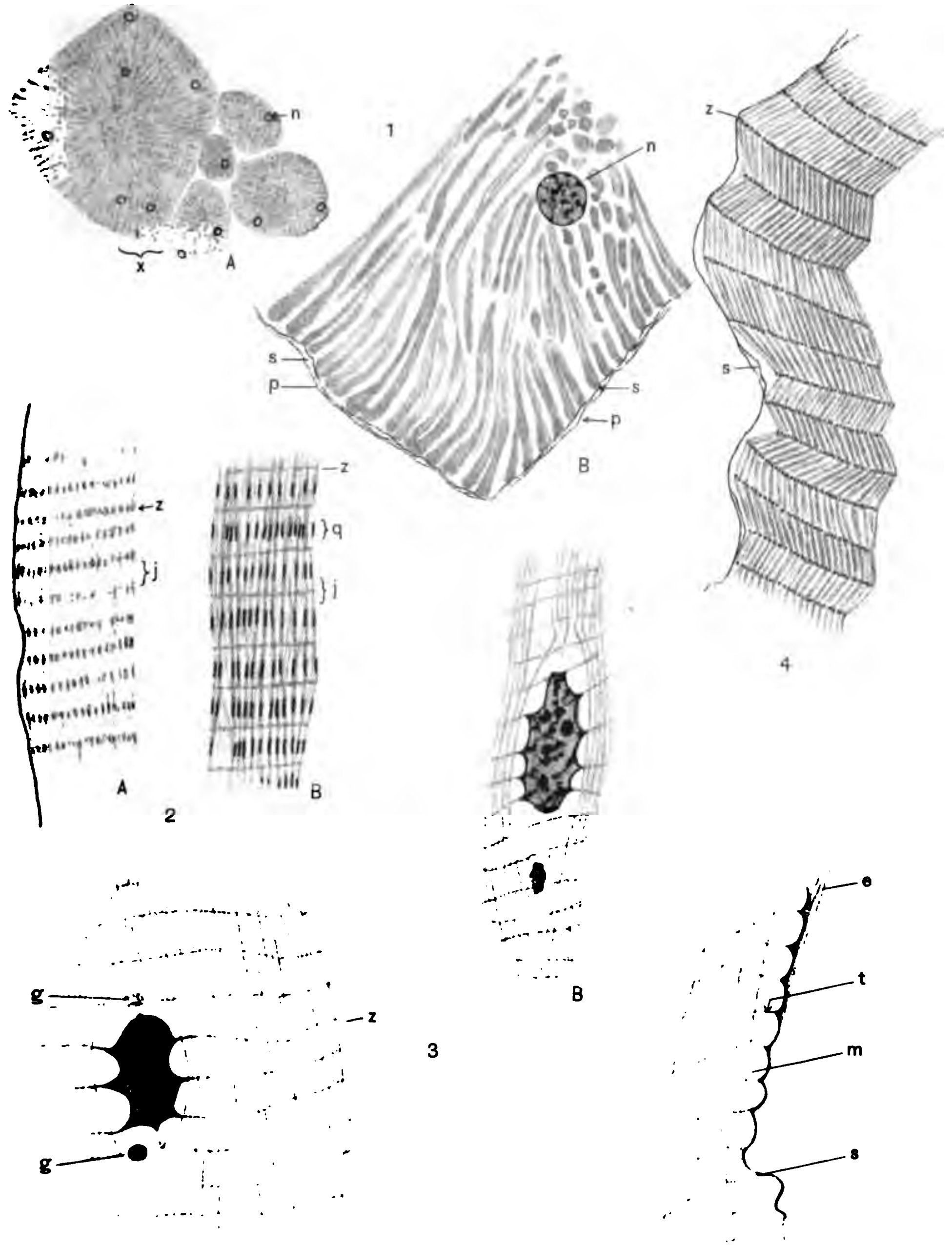
Fig. 12.—Heart-muscle nuclei (A and B) showing the relationship of the telophragmata to the nuclear wall. The perinuclear sarcoplasm is bounded by fibrillæ; there is no indication of a cell-membrane. The nuclear wall projects into spines at the points where the ground-membranes are attached. ×2,000.

Fig. 13.—Two adjacent cardiac fibers with intervening connective tissue (*c. t.*, endomysium). *R*, uncontracted fiber; *C*, contracted fiber; *S*, sarcolemma; *b. c.*, blood-cell. Note the difference in size and structure between the nuclei of the connective tissue and the muscle tissue. Both connective-tissue cytoplasm and the perinuclear sarcoplasm contain lipoid spherules. ×2,000.

Fig. 14.—Multinucleated sarcoplasmic area. The division process is amitotic. ×2,000.

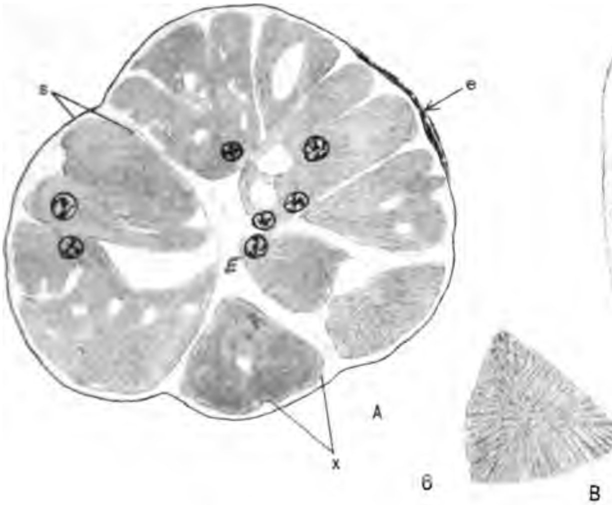
Fig. 15.—Intercalated disk of cardiac-muscle fiber of *Limulus*, dividing a contracted (upper) portion from an uncontracted (lower) portion. ×2,000.

Fig. 16.—Diagrams to illustrate the probable derivation of the complex serrated type of intercalated disk characteristic of hypertrophied muscle from the simple-comb type by process of longitudinal splitting of the myofibrils and unequal tensions among adjacent fibrils. The myofibrils are numbered 1 to 5; *Z*, telophragma; *d*, intercalated disk, (A) comb type, (B) type found sparingly in mammalian hearts, and exclusively in hypertrophied hearts. The distortions suffered by the successive telophragmata adjacent to the one directly involved in the disk are not taken into account in the diagram B.

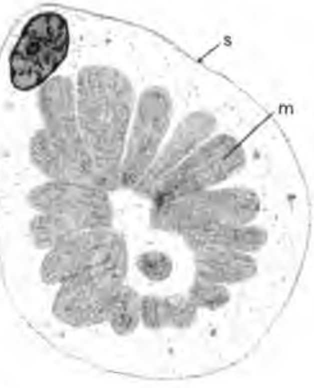


A Horn & Co Lith.

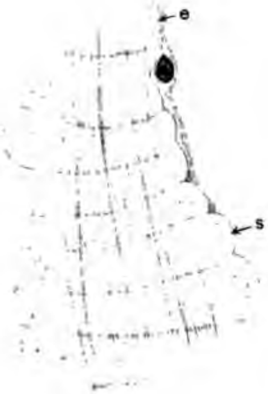
LIMULUS SKELETAL (SPINE) MUSCLE



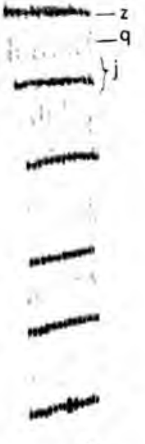
6



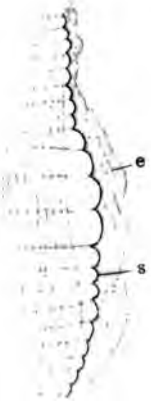
7



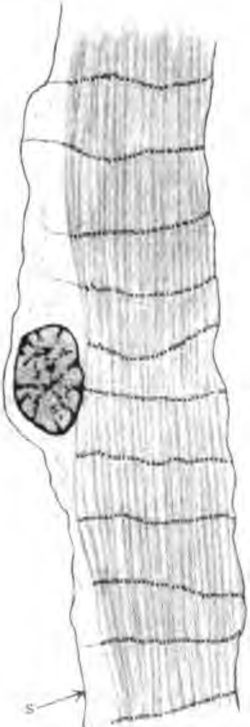
8



9



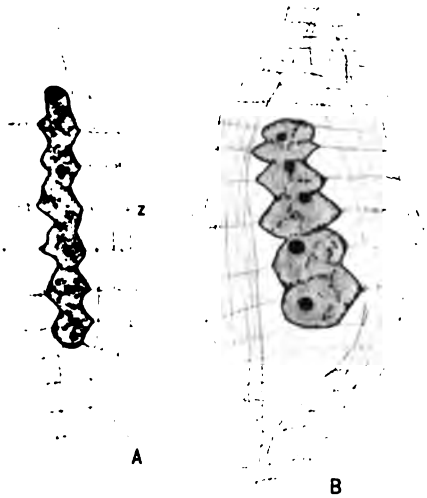
10



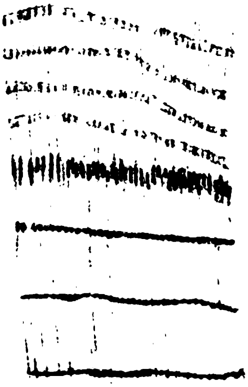
11

A Hoen & Co. Lith.

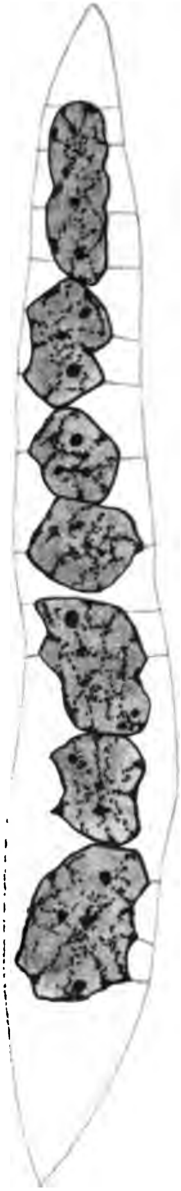
LIMULUS CARDIAC MUSCLE



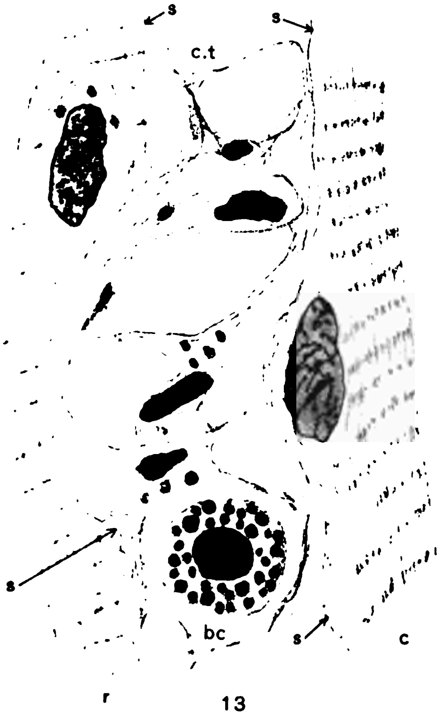
12



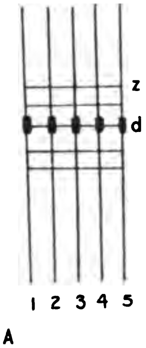
15



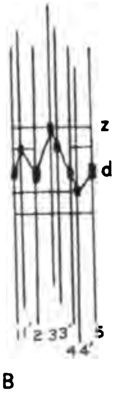
14



13



16



A. Hoen & Co. Lith.

LIMULUS CARDIAC MUSCLE

XII.

HEMOPOIESIS IN THE MONGOOSE EMBRYO, WITH SPECIAL REFERENCE TO THE ACTIVITY OF THE ENDOTHELIUM, INCLUDING THAT OF THE YOLK-SAC.

BY H. E. JORDAN,
Department of Anatomy, University of Virginia.

Five plates of twenty-seven figures.

HEMOPOIESIS IN THE MONGOOSE EMBRYO, WITH SPECIAL REFERENCE TO THE ACTIVITY OF THE ENDOTHELIUM, INCLUDING THAT OF THE YOLK-SAC.

By H. E. Jordan.

INTRODUCTION.

In several recent papers dealing with hemopoietic phenomena in turtle (7) and in pig embryos (8 and 9) I described appearances on the basis of which I maintained a contributive rôle on the part of the endothelium, both intraembryonic and yolk-sac. This position is in agreement with that of Schridde (20), Maximow (11 and 12), and others who have studied the origin and development of blood-cells in various forms. Principally in the work of Maximow and of Dantschakoff (3) this method of partial blood-cell origin has become related to the monophyletic theory of hemogenesis which they support. On the basis of his experimental work with *Fundulus* embryos, narcotized with alcohol, Stockard (21) concludes that the endothelium in these embryos can not transform into hemoblasts; and he views with skepticism the whole mass of morphologic evidence offered in proof of the hemogenic capacity of endothelium, claiming that a different interpretation of descriptions and illustrations is at least as plausible as the one usually given. Stockard, moreover, attacks the monophyletic theory, and in the development of his argument brings the non-hemogenic rôle of endothelium into relation with the polyphyletic theory of blood-cell origin. Moreover, those who believe in the strict specificity of endothelium, and in a degree those who accept the angioblast theory of His, dispute the possibility of endothelium to give origin to blood-cells. It is the chief purpose of this contribution to state and illustrate the evidence which in the opinion of the author justifies a belief in the endothelial origin of some hemoblasts and which agrees to a considerable extent with a monophyletic interpretation of hemopoiesis. This study confines itself largely to the mongoose embryo. No special virtue is claimed for this form in this regard. The chief value of this material lies in the fact of a superb fixation and a favorable staining, and in that it is of a stage of development (5 to 7 mm.) where the phases in question are especially abundant and clear. Moreover, it serves well as a key to the proper interpretation of certain aortic cell-clusters described for the 10-mm. pig embryo. (Emmel (4 and 5); Jordan (9).)

MATERIAL AND METHODS.

The material includes three embryos, with yolk-sac attached, of 5, 6, and 7 mm. length. The embryos were collected in March 1912 at Montego Bay, Jamaica, British West Indies, while with the scientific expedition of the Department of Marine Biology of the Carnegie Institution of Washington, under the leadership of the Director, Dr. Alfred G. Mayer. The embryos were fixed in Helly's fluid, stained *in toto* with Delafield's hematoxylin, lightly counterstained with eosin, and sectioned in paraffin at 10 microns. From the viewpoint of hemopoietic phenomena the three embryos are practically identical. Since the 5 mm. embryo seems to possess a slight advantage in respect of abundance of crucial stages and of differential staining, the following description will pertain almost exclusively to this embryo. The tissue is perfectly normal, as is indicated by the abundant mitoses in practically every tissue. The cell-clusters of the aorta show a progressive increase in size and differentiation from the 5 to the 7 mm. embryo.

HEMOPOIESIS IN YOLK-SAC.

The yolk-sac wall consists of three layers: (1) the thin superficial mesothelial layer; (2) the wide middle mesenchymal layer, filled with endothelium-lined blood-channels; (3) the inner entodermal lining, consisting of a single layer of large cuboidal cells more or less flattened (fig. 2, plate I; and fig. 23, plate III).[1]

The entodermal cells are characterized by the typical cytology described for those of the 10-mm. pig embryo—large vesicular nucleus and a granulo-alveolar cytoplasm frequently containing long, delicate basal filaments. The cytologic evidence indicates a secretory function. A number of the cells are in mitosis.

The mesothelium consists of greatly flattened cells with long oval vesicular nuclei. The mesothelium is in syncytial continuity with the middle mesenchymal layer. Where mesothelium and endothelium abut the two tissues become continuous, and no differential marks, either nuclear or cytoplasmic, appear to identify the two. Cells of either tissue may have larger or smaller nuclei, more chromatic or less chromatic, with a delicately or coarsely granular reticulum, and a more deeply or less deeply staining cytoplasm, depending probably upon the particular phase of modification or function. Exactly the same description will hold also for the mesenchyma. Except for a frequently stellate shape of the mesenchymal cells, the three tissues—mesothelium, endothelium and mesenchyma—are structurally practically identical at this stage. Mesothelium and endothelium are derivatives of the mesenchyma, apparently under the operation principally of the mechanical factor of pressure.

---

[1] The photomicrographs were made by Mr. William S. Dunn, Cornell University Medical School, New York.

The study of the origin of the blood-cells is best approached by a classification of the different types of cells found free in the blood-vessels, both yolk-sac and intraembryonic. The preponderating type of cell is the erythroblast with pale homogeneous cytoplasm and a spherical vesicular nucleus. The nucleus generally contains one or several nucleoli and a delicate, more or less granular chromatic reticulum (fig. 2). Many of these cells are in mitosis; an occasional cell shows a nuclear condition suggestive of amitosis (fig. 2). The homogeneous cytoplasm has a slightly acidophilic staining reaction; these cells correspond to those of the pig embryo, which, in Giemsa-stained material, have a grayish-pink color. An occasional cell has a slightly larger size than the average of the eythroblasts and is characterized by a finely granular cytoplasm, slightly acidophilic (fig. 12*b*). This is a still younger erythroblast, and corresponds to Maximow's megaloblast type. The granules are more probably the initial hemoglobin content. These cells are transition forms between basophilic hemoblasts and acidophilic erythroblasts. A few cells occur which are characterized by a denser, deeper-staining nucleus and a more highly acidophilic cytoplasm; these represent more differentiated erythroblasts and may be called normoblasts; no non-nucleated erythroplastids are yet present.

Hemoblasts also appear in considerable numbers, more abundantly in the yolk-sac vessels (fig. 1, *a* and *b*). These are characterized by a relatively large granular and vesicular nucleus, usually with several large chromatic nucleoli and a relatively thin shell of basophilic, apparently homogeneous, cytoplasm. In this material not a single mitotically dividing hemoblast was seen. In the pig embryo also a mitotically dividing hemoblast was an extremely rare occurrence. In both instances, however, nuclear amitotic phenomena are abundant. The initial stage is characterized by a kidney-shaped nucleus. The hemoblast may take very irregular shapes (see figure 1*b*) indicating amœboid activity. Figure 1, *c* and *d*, shows two late differentiation stages of a hemoblast; the nucleus stains less intensely, and the cytoplasm has become less basophilic. Figure 1*d* is binucleated, probably the result of a direct division of the nucleus. Trinucleated hemoblasts also are of frequent occurrence.

Giant cells are frequently met with. These differ from the hemoblasts apparently only in their greater size and the irregular character (fig. 1*e*) or the multiple condition of the nucleus. The size relation between nucleus and cytoplasm is slightly altered in favor of the latter. The cytoplasm is basophilic and the nucleus generally stains as intensely as that of the mononucleated hemoblast. The giant cell is most probably a hypertrophied hemoblast. Its multinucleated condition results from amitotic division of the hemoblast nucleus. The multinucleated forms are much less abundant in the 5-mm. mongoose embryo than in the 10-mm. pig embryo. Moreover, in the latter they have attained a much larger size and may contain many more nuclei than in the former. The evidence from the pig embyro suggested that giant cells were multiple erythroblasts, eventually differentiating into definitive erythrocytes. The giant cells of the mongoose include only the earliest stages of those described for the pig, hence no confirmatory evidence of the above conclusions regarding their erythropoietic function accrues from a study of these cells; but the evidence, so far as it goes, accords with that derived from the study of giant cells of the pig, and the tentative conclusion that they are erythroblasts rather than erythrophages may remain unaltered.

A careful study of the mesenchyma shows that numerous cells, both singly and in groups, take on nuclear and cytoplasmic hemoblast characteristics, and eventually round up more or less and separate from the parent mesenchyme. Exactly similar conditions were described and illustrated for the yolk-sac of the 10-mm. pig embryo and need not be further considered. As single cells these hemoblasts may wander into adjacent vessels; or as groups (blood-islands) they may become inclosed in endothelium forming in the surrounding mesenchyma, to produce an "angiocyst" of the growing vascular net. Similar conditions have frequently been described by various authors (Maximow, Dantschakoff, and others) in sections of the yolk-sac of various forms. Since Stockard has more recently described comparable processes in the yolk-sac of the living *Fundulus* embryo, there need remain no further doubt that mesenchyma does actually differentiate directly into hemoblasts and into enveloping endothelium.

The point of controversy now centers on the question whether the endothelium of the vascular net of the yolk-sac and elsewhere can transform into hemoblasts; for in the *Fundulus* embryo Stockard claims that the endothelium has no hemogenic capacity; and on the basis of this fact he casts doubt upon evidence contributed as a demonstration for blood-cell origin from endothelium in other forms (Schridde, Maximow, Jordan and Flippin, Jordan, Dandy, and others). However, Reagan's (18) more recent findings in *Fundulus* embryos contradict Stockard's conclusions on this point.

Appearances in the yolk-sac of the pig seemed to permit of no escape from the conclusion that endothelium did in fact transform in part into hemoblasts (8). The yolk-sac of the mongoose gives exactly the same evidence. This may be seen by a glance at figure 2*a*, 2*b*, or 2*c*. The cell *b* is at the crucial stage of metamorphosis. It is still continuous with the endothelium, and directly continuous with the endothelial cell *f*; but it has all the nuclear and cytoplasmic characteristics of a hemoblast (compare with 1*a* and 1*b*). Cell *c* represents a further stage in the same process and is about to separate from the endothelium as a mononculeated giant cell. A final stage is represented in figure 3, where a metamorphosed endothelial cell has just become separated, and has not yet fully rounded up into the typical hemoblast. Similar examples might be multiplied indefinitely; more are given in the paper dealing with this same phenomenon in the pig embryo; but enough has been shown, especially in the case of cell *b*, to leave no further doubt, I believe, that young endothelium can indeed transform into cellular blood elements.

It is of interest and importance to note that the origin of hemoblasts from endothelial cells also in the bone marrow, both under normal and certain pathological conditions, is described by some of the leading pathologists, *e. g.*, Aschoff (see Mallory's "Principles of Pathologic Histology"). Such a "lining cell" of the marrow blood spaces is believed by Aschoff and by Mallory to be capable of differentiation either into an erythrocyte, a granuloblast, or by hypertrophy into a megakaryocyte, which accords with the evidence derived from my study of the yolk-sac vessels of the pig and the mongoose. Moreover, Mallory, in his list of normal cellular blood elements (p. 21), substitutes for the commonly described mononuclear leucocyte (transitional leucocyte) his "endothelial leucocyte." This cell he derives from "the endothelial cells lining blood, and to a less extent lymph, vessels by proliferation and desquamation. They also multiply by mitosis after emigration from vessels into the lesions."

Since few any longer doubt that young mesenchymal cells can transform on the one hand into certain blood-cells and on the other into endothelium, it seems *a priori* reasonable to suppose that the only slightly altered mesenchyma, the embryonic endothelium, can also occasionally transform directly into hemoblasts. The same thing should be true also, perhaps to a lesser extent, with regard to mesothelium, and for the same reason. According to Bremer (1) the mesothelium covering the body-stalk of a 1 mm. human embryo does in fact give rise to some extent to blood-cells.

The main purpose of this paper is to attempt to establish the thesis that young endothelium has a hemogenic capacity, by showing that intra-embryonically also conditions occur similar to those described for the yolk-sac. These conditions relate to the endothelial origin of hemoblasts, which through a close series of developmental stages can be traced into erythrocytes.

The transition to intra-embryonic conditions next to be described may be made by way of figure 4, which represents a binucleated elongated hemoblast, from a yolk-sac vessel, about to separate from the endothelium with which it is still in part intimately connected and from which it has undoubtedly differentiated. The binucleated cell is apparently also about to divide into two hemoblasts, thus consummating the amitotic process.

CELL-CLUSTERS OF THE AORTA.

The next evidence for hemogenic capacity on the part of the endothelium pertains to certain phenomena in the abdominal portion of the dorsal aorta, the so-called "cell-clusters." Similar clusters in mammalian embryos were first (1909) observed by Maximow (11) in the rabbit embryo.[1] Dantschakoff (3) had already reported comparable structures in the chick embryo. Minot (13) later (1912) described them also for human embryos between 8 and 10 mm. Emmel (4) subsequently (1915) studied more in detail these clusters in pig, rabbit, and rat embryos. At the same time I was making a detailed study of the aortic cell-clusters in the 10 to 12 mm. pig embryos, and had come to essentially the same tentative conclusion regarding their significance as Emmel, namely, that they were masses of hemoblasts differentiating from the endothelium. In the 10-mm. pig embryo these clusters are abundant and very large, some consisting of 100 or more cells; they are practically limited to the ventral portion of the aorta (fig. 24). Proximally they are in continuity with the endothelium and the subjacent mesenchyma. Peripherally they consist of typical hemoblasts and young erythroblasts. The former are characterized chiefly by a deeper-staining basophilic cytoplasm, the latter by a lighter-staining acidophilic cytoplasm. Centrally transition stages occur between endothelium and hemoblast. Occasionally the clusters contain a central core of only slightly differentiated endothelial cells. A number of the cells may be in mitosis, and some exhibit phases in nuclear amitotic division. The mongoose material is of special value in that it shows the earlier stages in the formation of these clusters and thus gives the key to their proper interpretation. The pig material showed a progressive size increase between the 5 and 12 mm. stages of development. The mongoose material shows the same thing. In the 7-mm. embryo the clusters are somewhat larger and the peripheral cells more differentiated than in the 5 mm. embryo.

Figures 5 and 6 show typical small clusters, the first from the ventrolateral wall of the aorta, the latter from the mid-ventral line. Figure 5 gives the appearance of a buckling of the endothelium into the lumen of the aorta, the peripheral cells of the invaginated area assuming hemoblast characteristics. In the cluster, figure 6, the peripheral cells have progressed still further along this line of differentiation, and the proximal pole shows an increase of amitotic proliferative activity and transition phenomena on the part of the endothelium. In the case of the larger clusters the subjacent endothelium has in some instances undergone considerable thickening, being frequently three layers thick.

The close association between the cluster and the endothelium (figs. 25, 26, and 27) through a portion showing a transition between endothelium and hemoblasts, coupled with proliferative activity on the part of the constituent cells, should render unnecessary any discussion as to whether these clusters may not be groups of hemoblasts deposited from the circulating blood and caused by pressure to adhere to the vessel wall. The latter interpretation was given by Minot (13) to these clusters in human and rabbit embryos, in opposition to Maximow (11), who described them in the rabbit as arising by the proliferation of the endothelium. Minot's objection to Maximow's original interpretation was based on his failure to observe either in the human or the rabbit embryos any continuity between the protoplasm of the endothelial cells and that of the "mesamœboids" of the cell-clusters, or any considerable number of mitotic figures in the endothelial cells in the neighborhood of the clusters; "and, finally, because the endothelial nuclei are differentiated, while the nuclei of the cells of the clusters are not differentiated." The mongoose material, however, shows a definite cytoplasmic continuity between endothelial cells and the proximal cells of the clusters. Moreover, while endothelial mitoses are rare in the neighborhood of the clusters, various stages in the amitotic division of endothelial nuclei are abundant. As in the case of certain other tissues of various forms undergoing rapid growth under certain conditions (*e. g.*, blastoderm of pigeon, Patterson; tendon cells of new-born mouse, Nowikoff, etc.), the endothelial cells may here proliferate by the amitotic mode. Finally, the nuclear differentiation of the subjacent endothelial cells is of a lesser degree, judged especially by the small size of the nuclei and their spheroidal shape than that of the more peripheral hemoblast transition elements, many of which contain oval and kidney-shaped nuclei. Further countervailing evidence to the position that the clusters are accretion products from the circulation is detailed in another paper (9) and need not be repeated here.

---

[1] Professor Van der Stricht informs me that he had observed aortic cell-clusters in bat embryos as early as 1899; but he claims that the cells of these clusters only differentiate into leucocytes.

The aortic endothelium produces not only cell-clusters which contribute hemoblasts and erythroblasts, but single cells also separate from the endothelium in the same manner as described for the yolk-sac. Such cells may appear at any point in the wall of the mesonephric portion of the aorta, though they are more likely to be in the ventral region. A group of two cells appears in the mid-dorsal line of the aorta, subjacent to the notochord. Figure 7 illustrates an endothelial cell along the lateral wall which has rounded up centrally and has assumed hemoblast features; proximally it spreads out in delicate processes for a considerable distance, and is still continuous with the endothelium. Figure 8 represents an endothelial hemoblast separating from the dorsal wall of the aorta. Figure 9 illustrates still another method of separation; this hemoblast is directly continuous on one side with the endothelium. In figure 10 is shown a 2-cell "cluster" of hemoblasts from the lateral wall of the aorta. Figure 11 illustrates a 3-cell group, located just to the side of the mid-ventral line. This group is still partially attached dorsally to the endothelium and may be regarded as a later step in the process of endothelial hemoblast formation from the yolk-sac shown in figure 4.

In figure 12 is illustrated a 4-cell group from the ventro-lateral wall. Three cells are arranged in horizontal series and jut out directly into the lumen of the aorta; the three cells, moreover, are at successively later stages of differentiation. The proximal cell has intermediate features between an endothelial cell and a hemoblast (*a*); the distal cell has erythroblast characteristics and is very similar to some of the adjacent aortic erythroblasts (*c*). Close to the proximal cell of this group the endothelium is thickened and contains an increased number of nuclei, similar to the attached pole of the multicellular clusters. For purposes of comparison, both dimensional and structural, a few of the adjacent cells of the lumen are shown. A series of successive stages of differentiation is indicated by the letters *a* to *d*; *a* is a typical hemoblast; *b*, a typical megaloblast; *c*, typical normoblasts, and *d*, a late erythroblast (normoblast) stage.

From the foregoing it seems clear that the endothelium of the aorta in the mesonephric portion may proliferate locally in the ventral or latero-ventral or even dorsal wall, giving rise ventrally to cell-clusters which grow in size, the constituent cells of which undergo a coincident differentiation into hemoblasts and young erythroblasts. Similar cell-clusters of larger size are nowhere to be found in the yolk-sac vessels; such are apparently not formed during the yolk-sac homopoiesis; this fact, if countervailing evidence were needed, would sufficiently discredit any interpretation of the aortic cell-clusters in terms of masses of hemoblasts carried to the aorta by the blood and caused to adhere to the ventral wall by reason of pressure and the adhesive properties of their cytoplasm. Coincident with the production of hemoblasts in the aorta through a cell-cluster phase, the endothelium of the same portion of the aorta at any point, though more generally ventrally, may produce also, through the transformation of individual endothelial cells, hemoblasts in an exactly identical manner as above described for the vessels of the yolk-sac. These two processes—hemoblast production by endothelial cell-clusters and by transformation of individual endothelial cells—are essentially similar; the difference involved is one of the degree of endothelial proliferative and differentiative capacity, not one of kind. A few of the cells of the larger cluster appear to have also phagocytic properties.

The question arises as to why the hemogenic activity of the aortic endothelium is generally limited to the ventral area of the mesonephric portion. Why do the larger clusters form only ventrally? This portion differs from other portions in that this is the region along which the larger ventral arterial blood-vessels to the abdominal viscera migrate caudally to their definitive location, the process involving a shifting of the larger stems (celiac artery, superior and inferior mesenteric arteries) from higher to lower ventral connecting segmental twigs. This region would seem to contain a less highly differentiated type of endothelium—that is, one of greater proliferative capacity, providing thus for an unequal growth between the dorsal and ventral walls. Herein may possibly reside the capacity for some of these endothelial cells to differentiate into hemoblasts. If this interpretation is correct, then the younger endothelium of the intra-embryonic vessels should also be able to transform into hemoblasts. Such youngest type of endothelium should be present in the numerous small blood-channels in the anterior head region next the brain.

ENDOTHELIAL HEMOGENESIS IN INTRAEMBRYONIC MESENCHYMA.

Search for evidence in support of this hypothesis is abundantly rewarded. Figure 13 represents a blood-channel which has just formed out of the pericerebral mesenchyma. The lumen of the channel still contains strands of dissolving mesenchyma. The four cells present represent four different stages in the transformation of a mesenchyme cell into a hemoblast, leading through an endothelial cell. Cell *a* is still typically mesenchymal, but is clearly in process of transformation into an endothelial cell, for two of its processes already form portions of the wall continuous with that portion formed by the typical endothelial cells *b* and *c*. The original endothelial cell now represented by *c* and *d* apparently divided its nucleus, thus forming a binucleated cell; the distal portion of this cell underwent differentiation into a typical hemoblast, while the proximal end represents a transition stage between a typical endothelial cell and a true hemoblast. In this single section of a small area, including four nuclei, the whole process of mesenchymal and endothelial hemogenesis may be seen in abbreviated form; and the process is essentially identical with the manner in which mesenchyma and endothelium function hemogenically in the yolk-sac wall.

Figure 14 is of a cross-section of a capillary vessel including a single endothelial cell, and with a diameter just sufficient to accommodate a single erythrocyte. Since this section is from the same vascularizing area as that of figure 13, the most probable interpretation that suggests itself is in terms of cells *c* and *d* of figure 13. If cell *c*, still continuous with the general mesenchyma, had differentiated into an endothelial cell, and *d* into an erythrocyte, we would have exactly the condition shown in figure 14. The endothelial wall here is still continuous with the mesenchyma, as shown by the strands of protoplasm at the upper pole. If the cytoplasm immediately enveloping the second nucleus of an originally binucleated cell differentiated into hemoglobin-containing protoplasm and thus into an erythrocyte, while the more peripheral cytoplasm remaining in functional association with the second nucleus differentiated into endothelial protoplasm, the actual condition represented would be realized. A similar complex of erythrocyte and encapsulating endothelium has been seen also in a larger endothelium-lined vascular space.

In figure 15 is shown a hemoblast separating from, but still in cytoplasmic continuity with, the endothelium of a small pericerebral blood-space.

The suggestion has been made that what is interpreted as an endothelial cell rounding up and differentiating into a hemoblast is in reality only an endothelial cell in preparation for mitosis. This is a plausible objection to the interpretation above given and must be met. I have studied many dividing endothelial cells in the pericerebral mesenchyma with this point in mind. It is a fact that both mesenchymal and endothelial cells acquire a deeper-staining cytoplasm just before mitosis; but a mesenchymal cell divides without rounding up and has a relatively smaller nucleus and a less coarse and chromatic nuclear reticulum; moreover, the cytoplasm invariably has a slightly less deep-staining reaction. In these preparations the color of the dividing mesenchymal cell is a deep pink, that of the differentiating hemoblast a brownish or bluish red. The same color difference obtains between the dividing and the hemogenic endothelial cell. Moreover, the intravascularly dividing endothelial cell contracts only relatively slightly, thus becoming a more or less stout spindle-shaped cell (figs. 16 and 18), but does not round up in typical hemoblast fashion. In figure 18 is shown a long dividing endothelial cell at the late anaphase stage; this more probably represents a lateral sprout from the main vessel which is cut in cross-section, but it shows the typical stout-spindle character of the dividing endothelial cell in contrast to the shorter and more spheroidal condition of the differentiating endothelial hemoblast.

The endothelium of these pericerebral vascular channels has considerable proliferative capacity, both internal and external (fig. 17). Extravascularly dividing endothelial cells round up more like hemoblasts and may indeed be progenitors of extravascular hemoblasts; but certain minor morphologic differential characters suggest that we may here be possibly dealing with simply slightly modified proliferating mesenchyma.

It appears, then, that young endothelium, whether in the yolk-sac, ventral area of the mesonephric portion of the aorta, or in the intra-embryonic mesenchyma (pericerebral), functions in the formation of hemoblasts, and in an essentially similar fashion.[1]

---

[1] The following recent observations have a special bearing upon this point: Huntington (Amer. Jour. Anat., vol. 16, p. 290, 1916) records for certain mammalian embryos that "other red cells develop by the direct transformation of the border endothelial cells lining the early lymphatic spaces." Reagan (Anat. Rec., vol. 10, p. 111, 1915) states that in "chemically treated teleost embryos" (*Fundulus heteroclitus*), "both the endocardium and myocardium have in this region become completely transformed into strongly eosinophilous erythroblasts." Certain investigators have described peculiar mesodermal "fiber cells" in *Limulus* and in spiders and scorpions which on the one hand transform into striped muscle, and on the other into "a special type of blood-corpuscle" or perhaps true blood-cells. (Patten, The Evolution of the Vertebrates and their Kin, p. 235.)

From the above there seemed to be indicated a hemogenic capacity also on the part of the endothelium of the mesonephric glomerulus. This region was carefully studied. But there is apparently no intravascular differentiation of endothelium into hemoblasts. An occasional cell has the appearance of being at an early phase of separation from the vascular endothelium; but in consequence of the irregularity of the plane of section, due to the delicate nature and the contorted condition of the glomerular capillaries, a confident interpretation seems impossible. The normal hemogenic capacity of the glomerular endothelium is certainly very meager, if not actually *nil.*

In an earlier paper (8) I stated the conclusion that in the pig embryo the glomerular endothelium liberated cellular elements (hemoblasts) extravascularly. Study of the sections of the mongoose embryo (of relatively younger stages of development) compels a revision of this conclusion. In the mongoose embryo the visceral layer of the Bowman's capsule of the mesonephric tubule, while closely applied to the glomerulus, is nevertheless easily distinguishable from the endothelium of the glomerular capillaries. The endothelial cells are flat plates; the cells of the capsule are cuboidal or pyriform in shape. An occasional cell appears between the two layers. This may be an endothelial cell separating extravascularly, but such interpretation must remain uncertain.

In the pig embryo the capsular epithelium is still more closely applied to the glomerulus, and the cells of both layers are very similarly flattened. Generally the endothelial nucleus is more vesicular than that of the capsule cell. The cells of the capsule are frequently rounded up and appear to be in early stages of separation. These are the cells which I interpreted as endothelial elements separating extravascularly; but in the light of conditions in the mongoose embryos, this conclusion does not seem warranted. In the mongoose embryo the vascular endothelium of the mesonephric glomerulus is apparently hemogenically inactive; but the presence of the pyriform cells in the capsular membrane (fig. 22), certain of which remain attached by the merest thread of protoplasm—coupled with the fact of an occasional free cell within the lumen of the capsular portion of the mesonephric tubule—suggests that certain of these cells may separate in the embryo to become macrophages. A similar process is described by Mallory in the case of certain infections of the human kidney (*e. g.*, acute capsular glomerulo-nephritis).

It is probably incorrect to regard the endothelium of the mesonephric glomerular capillaries as "embryonic" and relatively undifferentiated. This endothelium has to perform a specialized secretion process in connection with the nephric function of the mesonephros. This may explain the inability normally to produce hemoblasts. On the other hand, the capsular portion of the tubule plays a lesser rôle in this secretory process, and in the mongoose embryo is relatively much less highly differentiated, as indicated by the still cuboidal character of its epithelium. This fact, in view of the mesenchymal origin of this portion of the tubule, may underlie the possibility of a capsular cell to separate from its epithelium and become a free intratubular element, perhaps a macrophage.

CELL-CLUSTERS OF THE PERICARDIUM.

In view of what was said above, regarding the close primary relationship between mesenchyme, endothelium, and mesothelium, it seemed reasonable to expect that mesothelium also might to some extent differentiate hemoblasts, possibly leucoblasts. Careful examination of the pericardium reveals patches of proliferating and differentiating mesothelial cells (fig. 19) very similar to the cell-clusters described for the aorta (compare figs. 6 and 19). These clusters appear both on the visceral and parietal pericardium; and groups of similar cells (syncytia) occur also in the pericardial cavity (fig. 21). The latter most probably are separated portions of the pericardial cell-clusters. Also, single cells may apparently separate (fig. 20) in a manner very similar to that described for the aortic endothelium. All of these cells derived from the pericardium again have many features in common with hemoblasts. It seems quite probable that in the case of the pericardium we are dealing with sources of extravascular hemoblasts (leucoblasts) from the cœlomic epithelium. In the adult it is known that phagocytic leucocytes (macrophages) may arise from the peritoneum, and the same condition may well prevail also in the embryo. In certain experimentally produced teleost hybrids Reagan (18) also describes the transformation of mesothelium into erythrocytes.

DISCUSSION.

The mesenchyma is a fundamental hemogenic tissue. Among its proximate differentiation products are endothelium and mesothelium. These represent originally mechanical rather than functional differentiation products. As such they might be expected to have retained the original differentiative capacity of the parent mesenchyma. The histologic facts above outlined seem to prove that such is actually the case. All the facts detailed are perfectly consistent with this interpretation.

The possibility of origin of hemoblasts from endothelium has become associated with the monophyletic theory of blood-cell origin and contradicts the idea of strict specificity of endothelium demanded by the angioblast theory of intra-embryonic vascularization of His. The opposed ideas of the origin and genetic relationship of the erythrocytes and the leucocytes are expressed in the monophyletic and diphyletic (polyphyletic) theories. It needs to be emphasized that the formulated processes are still largely in theory. The bulk of the best evidence, however, seems to be in favor of a monophyletic genetic method. The above observations are in accord with, and in a degree a support to, the monophyletic theory. The evidence shows that young endothelium and mesothelium are only slightly altered (mechanically) mesenchyma, and that the two tissues may in early stages give rise to hemoblasts. In the young embryo these hemoblasts differentiate into erythrocytes. The primitive leucocyte ("lymphocyte") is represented by this hemoblast; in later stages this hemoblast (leucoblast) may differentiate into the various types of granulocytes.

The monophyletic theory rests, then, essentially upon these three facts: (1) the origin of the early hemoblast from mesenchyme, now no longer disputed; (2) the differentiation of these hemoblasts into erythrocytes, also no longer disputed; (3) the identity, inferred from a very close structural similarity, of hemoblast and lymphocyte, which latter is regarded as the progenitor of the granulocytes. The third point is the one on which the discussion and dissension center. Granting the verity of the third point, it follows that the primitive leucocytes appear before the erythrocytes. Such an ontogenetic sequence is in accord with the principle of progressive differentiation and with the phylogenetic history—a very significant fact.

The sharpest attack upon the monophyletic theory of recent years has been made by Stockard (21), on the basis of his observations on *Fundulus* embryos; but his more definite and positive facts, namely, the origin of the erythroblasts, "leucoblasts," and endothelium from mesenchyma as seen in the living embryo are actually in accord with the monophyletic theory and furnish strong additional support. The observation upon which he takes foothold to launch his attack, namely, the segregation of erythropoietic and leucopoietic foci, is open to the criticism of misinterpretation with respect to the latter. At any rate, his illustrations of primitive "leucocytes" do not inspire confidence; the karyorrhexis shown would alone make one suspicious of degenerative phenomena, and their general appearance raises the question whether they are not actually (as also Reagan (16) suggests) degenerating erythroblasts suffering nuclear changes and hemolysis.

ADDENDUM.

The above was ready for publication in essentially the form here given early in March 1916. Meanwhile Emmel published a detailed description of the aortic cell-clusters in pig embryos (Amer. Jour. Anat., vol. 19, 3, 1916) and announced the publication of a paper "Concerning certain cellular elements in the cœlomic cavities and mesenchyma of mammalian embryos" (Amer. Jour. Anat., in press for vol. 19). After reading the first of these papers it seemed to me better to note and discuss them in an addendum than to rewrite my article on mongoose hemogenesis and attempt an incorporation of this discussion in the body of a revised paper. A glance at our illustrations shows the essential identity of our observations; and this point is emphasized by a comparison of our descriptions, after allowance is made for differences in form and degree of development. Our independent observations are in essential agreement, but our interpretations are wide apart. The embryonic derivation of certain cellular blood constituents from proliferating and metamorphosing endothelium seems well established; as is also the origin of leucocyte-like cells (macrophages?) in the serous fluids from the mesothelium of the serous cavities. I incline to view the whole process as a normal hemogenic phenomenon.

Emmel now adheres to a pathologic interpretation, concluding that, while vascular endothelium may not under normal conditions give rise to cellular elements of the blood, "it appears that in both embryo and adult mammals, endothelial tissue ordinarily passive may under certain abnormal conditions, however, assume proliferative activities contributing to the free cellular elements of the circulating blood"; also that "the participation of the mesothelium in the origin of macrophages in the embryonic cœlom is not improbably also a reaction to stimulative conditions arising in part at least through degeneration and disintergration of erythrocytes and other foreign elements escaping into these cavities."

Emmel evidently labors under a feeling of compulsion to interpret his findings in harmony with the original angioblast theory, which denies participation of the vascular endothelium in the normal process of the formation of cellular elements of the blood. It may be quite true that abnormal conditions of various sorts, experimental or pathologic, may stimulate the endothelium to proliferative activity, but recognition of this fact does not compel interpretation of all endothelial proliferative activity in terms of abnormal conditions.

That certain abnormal conditions do stimulate endothelium to proliferation and desquamation and a coincident differentiation proves only that endothelium carries the inherent capacity to thus behave. Similar behavior under early embryonic conditions may be a perfectly normal process. This similarity between a normal embryonic process and an abnormal later condition may be simply an aspect of a very widespread phenomenon in which a pathologic adult condition is an abnormally reawakened normal embryonic condition, *e. g.*, developing cardiac muscle and hypertrophying cardiac muscle, etc. A leiomyoma arises apparently as the result of a normal differentiation of smooth muscle-cells in an abnormal degree. Tumor cells are generally believed to be biologically of the same nature as normal cells. The cell-clusters above described would, if increased and enlarged to an abnormal degree, produce a condition comparable to a hemangio-endothelioblastoma.

Emmel's interpretation of the cause of the formation of free mesothelial derivatives appears especially strained. Why may not the mesothelium normally give rise to the cellular elements of the serous fluids as part of its function, by reason of its close genetic relationship to hemogenic mesenchyma and endothelium? At one point Emmel inclines (p. 402) to identify the cells of the cell-clusters of the aorta with macrophages, and he ascribes the cause of their formation to the presence of possible toxins produced by the "atrophying" and "degenerating" ventral aortic vessels in close association with which some of the cell-clusters are found. This conclusion is based upon a number of fundamental uncertainties. In the mongoose material the cell-clusters consist of typical hemoblasts (mesamœboids); this is in agreement with both Maximow's findings for rabbit and Minot's observations on human and rabbit embryos. The endothelial proliferation products of the embryo need therefore not be confused with macrophages. Moreover, Weidenreich derives the macrophages of Metschnikoff from leucocytes, while Evans avers that macrophages and leucocytes have no direct genetic relationship. On the other hand, many hematologists derive the leucocytes from the common blood mother-cell, the hemoblast, which may have an endothelial origin. A second uncertainty involves the manner of the caudal progression of the celiac, the superior mesenteric, and the inferior mesenteric arteries. Evans inclines to explain the process on the basis of an unequal growth between the dorsal and ventral portion of the abdominal aorta, necessitating thus a less highly differentiated endothelium ventrally. Degeneration of certain ventral vessels probably also occurs; but coincident with this atrophy there may be likewise a new origin of vessels in the formation of progressively lower connections with the main ventral stems; and some of the cell-clusters may be related to the newer vessels, as I believe, rather than to the degenerating vessels, as Emmel believes.

There appears no valid reason why the endothelium of the yolk-sac vessels and that of the embryonic vessels should function differently; why in the case of the yolk-sac vessels the endothelium should be capable of metamorphosing normally into hemoblasts, while in the intra-embryonic vessels with young endothelium the stimulative factor to metamorphosis should have to be a pathologic one. If endothelial hemogenesis in the yolk-sac increased in activity coincident with the degenerative processes of the sac, Emmel's interpretation might be more confidently accepted; but this is precisely where it fails. Endothelial hemogenesis is most active in the yolk-sac of the 10 to 12 mm. embryos when hemopoiesis is at its height in these vessels. Subsequently it decreases, and by the 25-mm. stage no separating endothelial cells can be found. Moreover, in the small vessels of the pericerebral region endothelial cells may occasionally separate to become intravascular elements. In short, wherever only slightly differentiated endothelium appears, blood elements may originate. Again, if abnormal conditions could explain endothelial proliferation and metamorphosis in embryos, then the experimental *Fundulus* embryos of Stockard, in which cessation of heart-pulsation produced stasis in the blood-vessels, and a consequent degeneration of some of the included cells, should show endothelial proliferative activity; but according to Stockard this endothelium is inactive.

Emmel lays much stress upon the connection between certain ventral aortic clusters and intra-arterial cell-masses. Vessels containing such masses are interpreted as "degenerating"; but the evidence that such are degenerating is unconvincing. In a 10-mm. pig embryo cut in transverse section I found a large cluster attached to one side of the mouth of the superior mesenteric artery. A similar condition prevails in the 5-mm. mongoose embryo. These vessels are not degenerating; if cut obliquely similar clusters might appear to be attached to an intra-arterial cell-mass filling the vessel. Emmel's section No. 6 (p. 419) is apparently obliquely cut. The "intra-arterial cell-mass" may be simply attached to one side of the mouth of the vessel. Such conditions occur in both the mongoose and pig embryo, where no sign of atrophy appears in the vessel itself. On the other hand, ventral branches which later disappear can be seen free of cell-clusters. In short, neither the degeneration nor the occlusion of all aortic branches associated with clusters is definitely proved.

Nevertheless, certain aortic branches undoubtedly do disappear in the caudal shifting of the ventral branches, and this process may be of the nature of a degeneration which may liberate a dilute and slowly acting toxic substance comparable to such substances as stimulate the production of endothelial leucocytes in certain pathologic conditions, *e. g.*, the relatively slightly virulent toxins arising from typhoid and tubercle bacilli. Then this effect should either be felt throughout the whole of the abdominal aorta, or there should be a progressively decreasing effect, as indicated by the abundance of the desquamating endothelial elements from the distal to the proximal (aortic) portion of these ventral rami. But neither of these conditions obtain. In the older embryos the clusters and desquamating cells are practically limited to the ventral wall; in the younger embryos (mongoose) where there are fewer atrophying ventral rami, desquamating cells can be found in the lateral and even the dorsal wall, and the ventral cell-clusters are small. The younger the embryo the less differentiated the endothelium. Moreover, the clusters of the older embryos are near the mouths of the vessels, or farthest removed from the site of presumed intensest degeneration; and numerous vessels contain no clusters at all.

In the 7-mm. mongoose embryo several occluded ventral rami appear, comparable to Emmel's figure 7. The lumen of the vessel is completely filled with hemoblast-like cells similar to those of the aortic clusters, many of which show degeneration stages, principally a karyorrhexis. But the presence of these cells need not be interpreted as the result of the liberation of a toxin by the atrophying ramus. It seems more reasonable to suppose that the ramus contained a cluster of normal hemoblasts as the result of a normal hemogenic capacity on the part of the endothelium of the vessel, which cluster, in consequence of the atrophy and coincident constriction of the vessel, came to occlude the lumen and ultimately to suffer a resultant degeneration. In other words, the occlusion of the lumen of the vessel and the karyorrhexis of the included hemoblasts are more probably secondary effects of the atrophy of the ramus than that the presence of the cells in the lumen is the result of a toxin formed by the degeneration of the vessels and the included cells and operating as a desquamating stimulant upon the endothelial cells of the vessel.

The most damaging countervailing evidence, however, to the interpretation that the endothelial desquamation products are the result of the action of toxins produced by degenerating blood-vessels and blood-cells, consists in the presence (in the 10-mm. pig embryo) of endothelial cell-clusters of hemoblasts, deep within the superior mesenteric artery (that is, in the middle third of its extent), a level which shows no other signs of atrophy or degeneration and which suffers no subsequent change in a possible farther caudal progression of the vessel to its definitive point of attachment to the aorta. Also in the mongoose embryos and in turtle embryos, the endothelium of the superior mesenteric artery is especially active in liberating intravascular cellular elements.

In a study of the aortic cell-clusters in loggerhead-turtle embryos my attention was arrested by peculiar formations in the inferior vena cava at the level of fusion of the subcardinal veins of a specimen of the twelfth day of incubation. These formations consisted of encapsulated spheroidal masses of hemoblast-like cells, and long strings of hemoblasts attached to the endothelium and extending free into the lumen of the vessel. In the case of the encapsulated groups the capsule consists of an endothelioid membrane of greatly flattened cells. The inclosed cells are very similar to those of the naked cell-clusters of the aorta and the superior mesenteric artery. The structure is comparable to Emmel's figure 5, which represents a similar formation in the aorta of a 12 mm. pig embryo. In one instance the mass was continuous with an underlying loose mesenchyma which appeared to be differentiating into hemoblasts. I incline to interpret this structure in terms of my figure 5. If the invaginated area of endothelium had included a considerable portion of the subjacent vascularizing mesenchyma, then the mesenchyma might have outstripped the endothelium in the process of differentiating into hemoblasts, and so forced, through pressure, the peripheral endothelial cells to continue their further differentiation into definitive endothelium.

As to the endothelial strands of hemoblast-like cells, I incline to an interpretation in accord with the idea that slightly differentiated endothelium anywhere carries the capacity of producing hemoblasts. In a footnote (p. 407) Emmel describes similar strands in the aorta and the proximal portion of the left umbilical artery in the 12-mm. pig embryo which contained the encapsulated cluster, and in a second 12-mm. embryo, and suggests that they may be associated with the fusion of the two original dorsal aortæ.

If one wishes to adhere to an interpretation of these proliferation products of the aortic endothelium in terms of a toxin, one might locate the source of the toxic substance in the mesonephros, where degenerative processes are initiated in the anterior portion; but such a view is again contradicted by the observation that endothelial desquamation products are practically lacking in the glomerular capillaries, though occasionally present near the aortic mouth of the afferent arteriole. The view that we are dealing with a normal hemogenic process related to a relatively undifferentiated condition of endothelium such as would seem to be requisite in the ventral portion of the abdominal aorta, to permit of the shifting of the celiac, superior mesenteric, and inferior mesenteric arteries by almost any reasonably conceivable process, seems to fit all the facts better than the idea of a toxic influence dependent upon a tissue degeneration in redundant atrophying blood-vessels.

In a second paper ("Concerning certain cellular elements in the cœlomic cavities and mesenchyma of the mammalian embryo," Amer. Jour. Anat., vol. 20, 1, 1916, pp. 73–125), Emmel describes the origin of "macrophages" from the pericardial and peritioneal mesothelium; he describes and illustrates also certain free cell-masses in the pericardial cavity comparable with my illustration (fig. 21) in the mongoose embryo. The causal factor is again assumed to be a toxic substance, in this case liberated in part by degenerating erythrocytes in the cœlom. But it would seem quite as reasonable to regard this formation of macrophages in the embryo as a normal incident in the life of the embryo.

In conclusion, I believe that a careful consideration of all the facts relative to Emmel's observations and my own, upon which there is essential agreement, more amply justifies the conclusion that young, relatively undifferentiated endothelium and mesothelium may anywhere in the embryo normally produce hemoblasts (and macrophages), than that such activity demands the operation of a pathologic factor in the form of a dilute, slow-acting toxin.

BIBLIOGRAPHY.

1. **Bremer**, J. L. 1914. The earliest blood-vessels in man. Amer. Jour. Anat., vol. xvi, p. 447.
2. **Dandy, Walter** E. 1910. A human embryo with seven pairs of somites, measuring about 2 mm. in length. Amer. Jour. Anat., vol. 10, p. 85; fig. 10, p. 105.
3. **Dantschakoff, Wera.** 1908. Untersuchungen über die Entwicklung des Blutes und Bindegewebes bei den Vögelen. I. Die erste Entstehung der Blutzellen beim Hühnerembryo, usw. Anat. Hefte, Bd. 37.
4. **Emmel**, V. E. 1915. The cell-clusters in the dorsal aorta of the pig embryo. Anat. Rec., vol. 9, p. 77 (Proc. Amer. Assoc. Anat.).
5. **Emmel**, V. E. 1916. The cell-clusters in the dorsal aorta of mammalian embryos. Amer. Jour. Anat., vol. xix, p. 401.
6. **Emmel**, V. E. 1916. Concerning certain cellular elements in the cœlomic cavities and mesenchyma of the mammalian embryo. Amer. Jour. Anat., vol. xx, p. 73.
7. **Jordan**, H. E., and J. C. **Flippin**. 1913. Hæmatopoiesis in Chelonia. Folia Hæmatologica, Bd. 15.
8. **Jordan**, H. E. 1916. The microscopic structure of the yolk-sac of the pig embryo, with special reference to the origin of the erythrocytes. Amer. Jour. Anat., vol. xix, p. 277.
9. **Jordan**, H. E. 1916. Evidence of hemogenic capacity of endothelium. Anat. Rec., vol. 10, p. 417.
10. **Mallory**, F. B. 1914. The principles of pathologic histology.
11. **Maximow**, A. 1909. Untersuchungen über Blut und Bindegewebe. I. Die frühesten Entwicklungsstadien der Blut- und Bindegewebszellen beim Saügetierenembryo, u. s. w. Arch. f. mikr. Anat., Bd. 73.
12. **Maximow**, A. 1910. Ueber embryonale Entwicklunge der Blutzellen bei Selachiern und Amphibien. Anat. Anz., Bd. 37 (Ergängzungsheft).
13. **Minot, Charles** S. 1912. Development of the blood. Chapter xviii, part 1, pp. 523–524, Human Embryology, Keibel and Mall.
14. **Nowikoff**, M. 1910. Zur Frage über die Bedeutung der Amitose. Arch. Zellf., Bd. v, p. 365.
15. **Patterson**, J. T. 1908. Amitoses in the pigeon's egg. Anat. Anz., vol. xxxii, No. 5.
16. **Reagan**, F. P. 1915. A further study of the origin of blood vascular tissues in chemically treated teleost embryos, with special reference to hæmatopoiesis in the anterior mesenchyme and in the heart. Anat. Rec., vol. 10, p. 99.
17. **Reagan**, F. P., and J. M. **Thorington**. 1915. The vascularization of the embryonic body of hybrid teleosts without circulation. Anat. Rec., vol. 10, p. 79.
18. **Reagan**, F. P. 1916. Experimental studies on the origin of intraembryonic endothelium and blood-cells. Anat. Rec., vol. 10, p. 235 (Proc. Amer. Assoc. Anat.).
19. **Schmidt**, M. B. 1892. Ueber Blutzellenbildung in Leber und Milz, usw. Zeigler's Beiträge, Bd. 11.
20. **Schridde**, —. 1908. Die Entstehung der ersten embryonalen Blutzellen der Menschen. Verhandl. d. deutsch. path. Gesellsch., Bd. 11.
21. **Stockard, Charles** R. 1915. The origin of blood and vascular endothelium in embryos without a circulation of the blood and in the normal embryo. Amer. Jour. Anat., vol. 18, p. 227; also Memoir No. 7, Wistar Inst. Anat. and Biol.
22. **Stockard, Charles** R. 1915. A study of wandering mesenchymal cells in the living yolk-sac and their developmental products: chromatophores, vascular endothelium, and blood-cells. Amer. Jour. Anat., vol. 18, p. 525.
23. **Stricht**, O. **Van der**. 1899. L'origine des premières cellules sanguines et des premières vaisseaux sanguins dans l'aire vasculaire de chauves-souris. Bull. de l'Acad. Roy. de méd. de Belgique, S. 4, T. xiii.

DESCRIPTION OF ILLUSTRATIONS.

All the illustrations, unless otherwise specified, are made from the 5 mm. embryo; the magnification is 1,600 diameters, which is reduced one-third in reproduction.

Fig. 1.—Hemoblasts from yolk-sac vessels: *a*, typical spheroidal type, with basophilic cytoplasm and deep-staining nucleus; *b*, similar cell in amœboid condition; *c*, slightly more differentiated type, with lighter-staining nucleus and cytoplasm (young erythroblast); *d*, binucleated hemoblast differentiated into a young erythroblast; *e*, giant cell (megakaryocyte) with deeply basophilic cytoplasm and irregular, horseshoe-shaped, deeply staining nucleus.

Fig. 2.—Portion of yolk-sac wall, including one large blood-channel fillled with erythrocytes. The mesothelium is shown on the left, the entoderm on the right; *a* to *d* designate successive stages in the metamorphosis of an endothelial cell into an erythrocyte. The cell at *b* has all the nuclear and cytoplasmic marks of a hemoblast, but it is still continuous with the endothelial wall. *c*, a very large hemoblast, also still continuous with the endothelium of the vessel.

Fig. 3.—Hemoblast just after separation from the endothelium.

Fig. 4.—A binucleated elongated hemoblast, still continuous with the endothelium and about to divide amitotically into two hemoblasts. The three large cells at the left are entodermal.

Fig. 5.—Cell-cluster arising in latero-ventral wall of mesonephric portion of aorta through proliferation and differentiation of the endothelium. The peripheral cells have hemoblast characteristics. 6 mm. embryo.

Fig. 6.—A similar small cluster from the ventro-medial portion of the aorta. Proximally the cluster is in syncytial continuity with the endothelium.

Figs. 7, 8, 9, 10.—Hemoblasts differentiating and separating from the lateral wall of the aorta.

Fig. 11.—A string of three hemoblasts, the cytoplasm of which is still in continuity, and which have differentiated from the endothelium of the latero-ventral wall of the aorta. The proximal hemoblast is still in continuity with the endothelium. 6 mm. embryo.

Fig. 12.—A string of three hemoblasts (*a*) jutting from the endothelial wall into the lumen of the aorta. Within the lumen are shown five erythrocytes; *b*, young erythroblast (megaloblast) with finely granular slightly acidophilic cytoplasm; *c*, normoblasts; *d*, adult erythrocyte. 6 mm. embryo.

Fig. 13.—Binucleated hemoblast differentiating from the wall of a pericerebral blood-channel. The proximal nucleus and its enveloping cytoplasm have endothelial characteristics, and are continuous with the endothelium, which is continuous above with the mesenchyma. The distal nucleus and its enveloping cytoplasm have hemoblast characteristics.

Fig. 14.—Pericerebral capillary with one erythrocyte. This condition may be conceived to have arisen from a binucleated cell as in figure 13, in which one nucleus and the peripheral portion of the cytoplasm differentiated into endothelium, the other into an erythroblast.

Fig. 15.—Hemoblast differentiating from the endothelial wall of a pericerebral vascular space.

Fig. 16.—Pericerebral blood-vessel in which an endothelial cell is dividing internally by mitosis.

Fig. 17.—Pericerebral blood-vessel upon which an endothelial (mesenchymal) cell is dividing externally by mitosis.

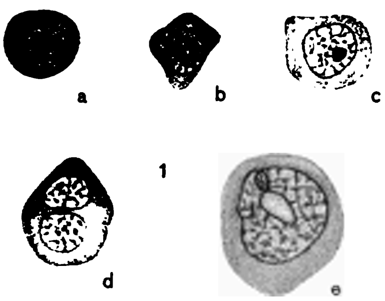
Fig. 18.—Pericerebral blood-vessel in cross-section (containing an erythrocyte) from one point of which is sprouting a lateral vascular twig. The sprouting endothelial cell is at a late anaphase of division. 6 mm. embryo.

Fig. 19.—Cell-cluster of the atrial visceral pericardium. These clusters are similar to those of the aorta, the constituent cells including some with hemoblast characteristics.

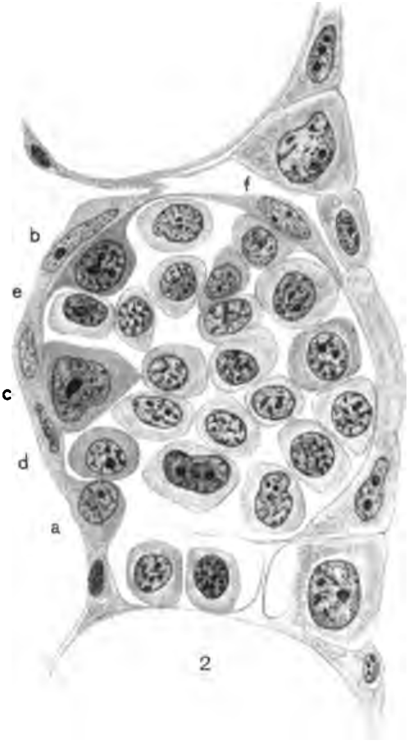
Fig. 20.—Hemoblast-like cell separating from the mesothelial layer of the aortic bulb.

Fig. 21.—Free cell-cluster from the pericardial cavity.

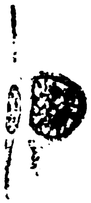
Fig. 22.—Portion of visceral layer of capsule of mesonepheric glomerulus, showing cells in process of separation.



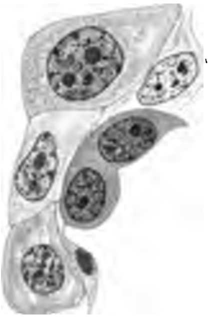
1



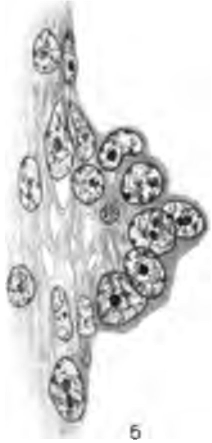
2



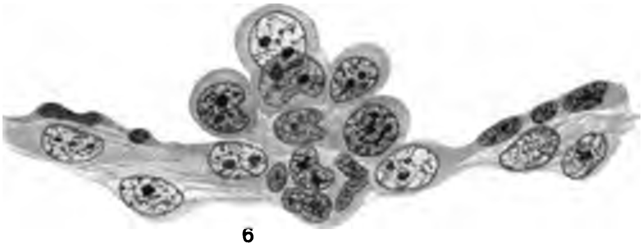
3



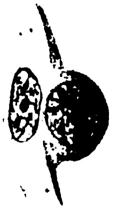
4



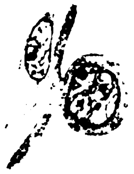
5



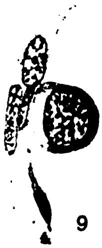
6



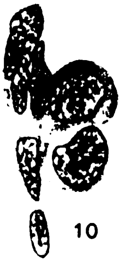
7



8



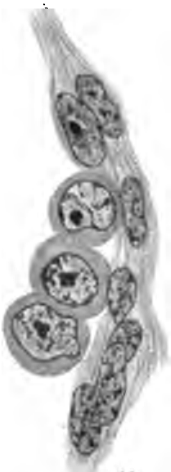
9

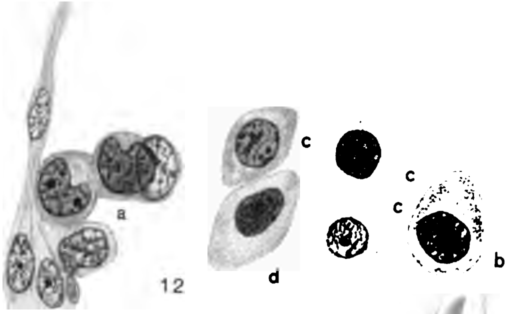


10

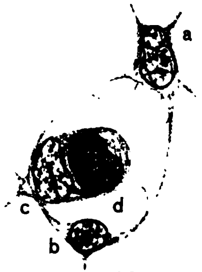
HEMOPOIESIS IN MONGOOSE

A.Hoen & Co Lith.

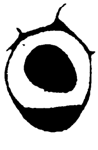

11

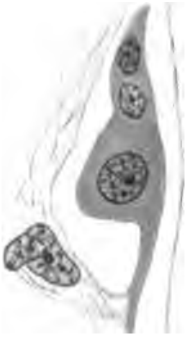


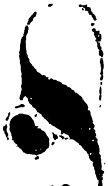
12

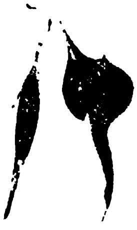


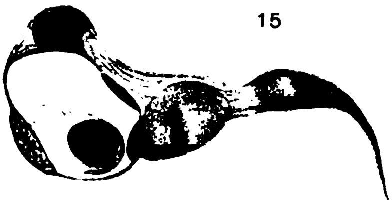
13

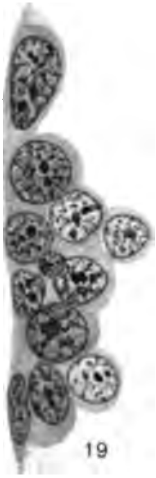

14

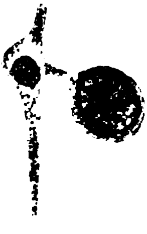

15

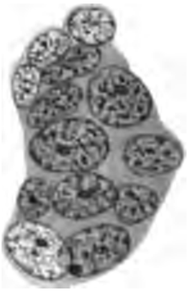

16

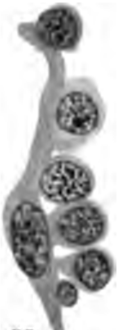

17


18


19


20

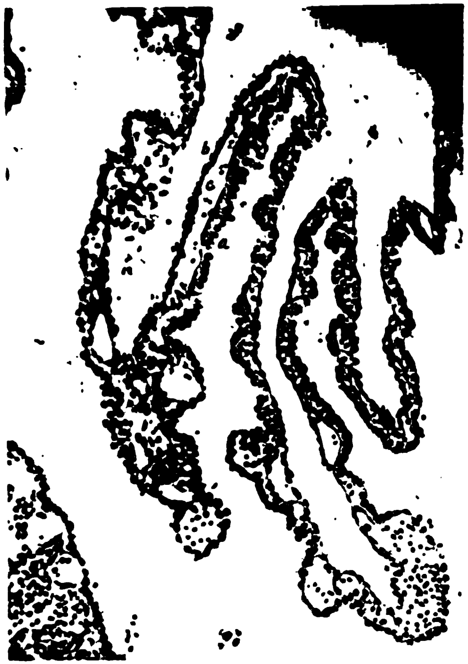

21


22

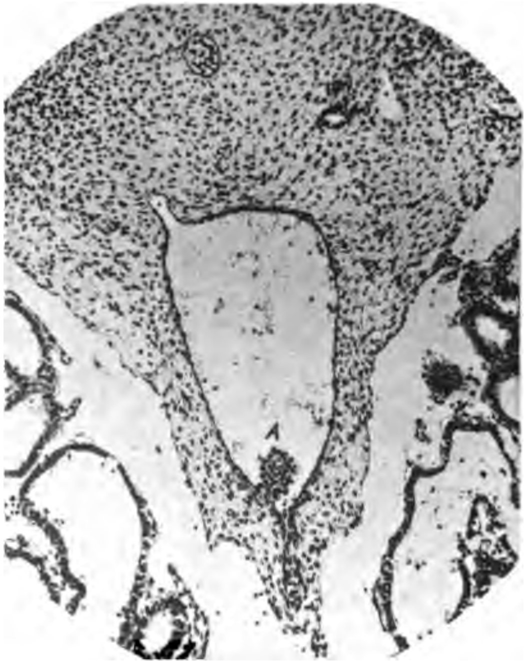
HEMOPOIESIS IN MONGOOSE

A Hoen & Co. Lith.

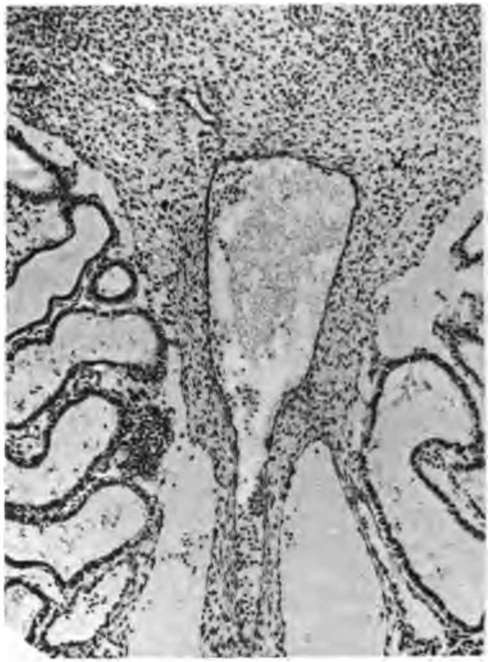
23



24



25

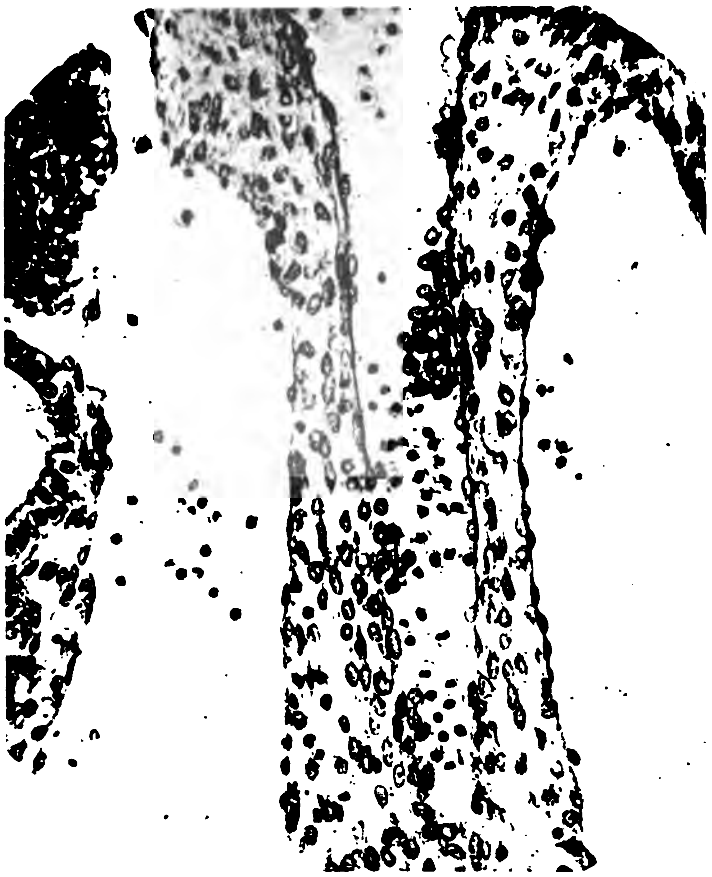


23 Section of portion of yolk-sac wall of 5 mm. mongoose embryo showing, especially well in central upturned loop, character and general relationship of the entodermal lining epithelium (*a*), covering mesothelium (*b*), and blood-space (*c*) filled with erythrocytes. Photomicrograph x 112.

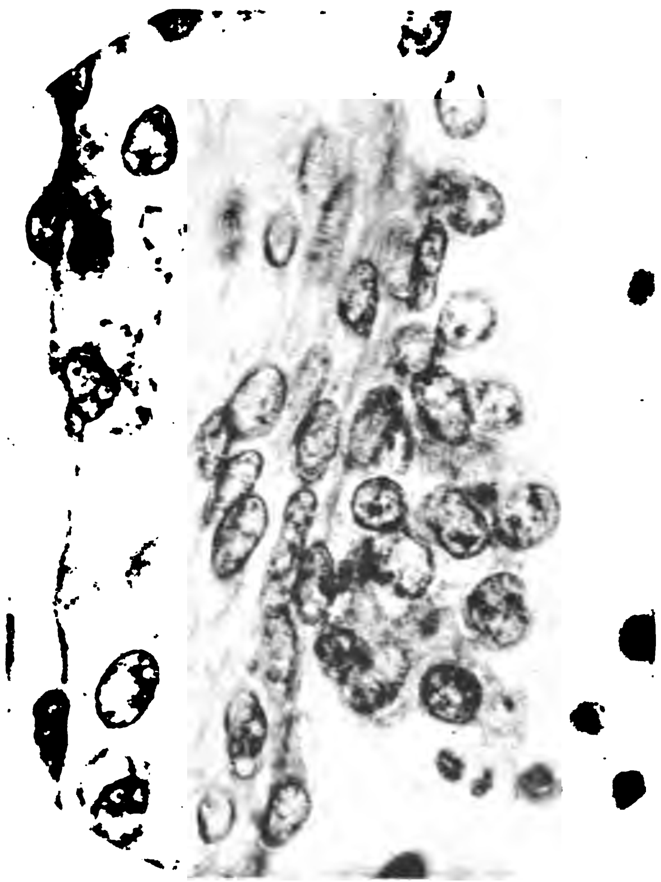
24. Portion of transverse section of a 10 mm. pig embryo at level of first ventral branch below the superior mesenteric artery, showing large cell-cluster (*A*) of hemoblasts and peripheral young erythroblasts within the aorta near the ventromedial line. The proximal cells of the cluster are in cytoplasmic continuity with the endothelium. This cluster extends through 130 microns. Ventral vessel cut tangentially through its caudal wall. The cluster is not in continuity with cells in the ventral branch, nor is there evidence of degeneration in this vessel. Note mesonephros on either side of aorta, and notochord above. Photomicrograph x 85.

25. Portion of transverse section of same embryo (360 microns forward of fig. 24) in the region of the definitive superior mesenteric artery, showing a cell-cluster within this vessel. Three sections caudalward the superior mesenteric artery is open for a length approximately 3 times that here shown. It contains within the middle third other smaller clusters attached to its wall, but there is no evidence of occlusion or degeneration. Photomicrograph x 100.

26



27



26. More highly magnified photomicrograph of cell-cluster of fig. 25, showing continuity of proximal cells of the cluster with the endothelium of the vessel, and the hemoblast character of the peripheral cells. Within the lumen of the vessel are shown numerous erythrocytes. x 230.

27. Cell-cluster of superior mesenteric artery shown in figs. 25 and 26. Photo x 500.

XIII.

EMBRYONIC HISTORY OF THE GERM-CELLS OF THE LOGGERHEAD TURTLE (CARETTA CARETTA).

By H. E. JORDAN,

Department of Anatomy, University of Virginia.

Six plates of twenty-four figures.

TABLE OF CONTENTS.

PAGE.
Introduction 315
Material and Methods 316
Descriptive 319
    11-day embryo; Flemming fixation, iron-hematoxylin stain 319
    10-day embryo; Helly fixation, Giemsa stain 322
    12-day embryo; Helly fixation, iron-hematoxylin stain 322
    Older embryos; from 13 to 32 days 323
    Younger embryos; from 9 to 6 days 325
    Youngest embryos; from 2 to 5 days 326
Literature 327
    Germ-cell origin in invertebrates 327
    Germ-cell origin in vertebrates 328
Discussion and Conclusions 333
Summary of Results 340
Bibliography 342
Description of Illustrations 344

EMBRYONIC HISTORY OF THE GERM-CELLS OF THE LOGGERHEAD TURTLE (CARETTA CARETTA).

By H. E. Jordan.

INTRODUCTION.

The wide discrepancies in the published accounts of the origin and early history of the germ-cells in vertebrates provided the stimulus for the inception and prosecution of this investigation. Thus Waldeyer (1870) described in the 4-day chick embryo the differentiation of germ-cells from the mesothelium covering the mesonephros. This portion of the cœlomic epithelium he designated "germinal epithelium." The theory of germ-cell origin from the mesothelium of the genital ridge early received the indorsement of Semper (1875). Semon (1887) also claimed to have seen a number of cells undergoing metamorphosis from "germinal epithelium" into germ-cells. This theory still forms the basis of the almost universal text-book account, and claims adherents among very recent investigators, *e. g.*, Gatenby (1916).

In 1880, Nussbaum, on the basis of observations on trout and frog embryos, advanced a rival theory which maintained a direct blastomeric origin of the germ-cells and an extra-regional segregation until relatively late stages in the histogenesis of the sexual gland. The central idea of this theory was later (1886) generalized in Weismann's hypothesis of "the continuity of the germ-plasm."

Rückert in 1888 described the origin of the "primordial germ-cells" from a portion of the segmental mesoderm, the so-called "gonotome." Minot (1894) analyzed the available evidence, but adjudged it inadequate to support this novel hypothesis.

In 1892 Hoffman published new evidence, derived from a study of the embryos of various birds, in support of Nussbaum's theory of a germinal path ("Keimbahn") in vertebrates. He developed further the idea that the germ-cells are early segregated as independent elements, *sui generis*, and that these find their way from among the cells of the entoderm to the cœlomic epithelium covering the genital ridge. An increasing number of investigators (*e. g.*, Eigenmann, 1892; Woods, 1902; Allen, 1906, 1907, 1911; Swift, 1914, 1915, 1916) are adding evidence in extension of Hoffman's observations and in accord with the segregation theory of Nussbaum.

In the chick, Swift (1914) traces the origin of the germ-cells back to the entoderm in a peripheral crescentic area in the proamniotic region of the primitive-streak stage, from whence they are described as migrating by way of developing blood-channels to the splanchnopleure and thence, by amœboid activity, to the primitive gonads.

In the lizard *Lacerta agilis*, von Berenberg-Gossler (1914) follows the origin of the so-called "primordial germ-cells" ("entodermal wandering cells"—Danchakoff, 1908) to the entoderm of the open gut; but he denies for them a direct genetic relationship to the definitive germ-cells, and regards the whole process as a late phase of mesoderm derivation from entoderm contributing largely to the formation of the mesonephric (Wolffian) duct.

Still others (*e. g.*, Felix, 1906, 1911; Dustin, 1910; Firket, 1914) admit the occurrence of primordial germ-cells in the entoderm and their migration in part to the developing genital gland, but attribute to this process only a phylogenetic significance, an ontogenetic reminiscence of an earlier phylogenetic experience; and they claim that these primitive sexual elements early degenerate and are replaced by secondary sex-cells which are differentiated from the mesothelium of the genital ridge.

In view of these discordant conclusions it seemed desirable that the scope of these investigations should be extended to include many more vertebrate forms and additional workers. Among the whole group of investigators Swift was the first to describe, or even to suggest, a vascular route of early migration. Von Berenberg-Gossler (1914) confirms this point for chick and for duck. The possibility seemed to remain, however, that these intravascular so-called genital-cells might indeed be only a type of blood-cell progenitor, perhaps a hypertrophied hemoblast. With this possibility in mind, suggested also by Minot's earlier tentative interpretation (1894) of these cells as elements enlarged in preparation for mitosis, and the later claim of Winiwarter and Sainmont (1909) that they are hypertrophied mesoderm-cells, the work on the loggerhead turtle embryos was planned.

It may be added also that my previous study of the early history of the female germ-cells of the starfish *Asterias forbesii* (1908) had led me to the conclusion that in this form the germ-cells most probably arose by differentiation from the peritoneal epithelium, and so inclined me to a belief in the essential accuracy and general applicability of Waldeyer's original theory. But a steadily augmenting body of cytologic and genetic data would seem to demand a continuous "germ-plasm," which demand does not appear to be met by a derivation of germ-cells from already differentiated soma-cells.

MATERIAL AND METHODS.

The first essential requisite was a complete series of embryos. The second requisite was a favorable staining technic, such as would clearly differentiate the primordial germ-cells at all stages of their early history; which condition was in turn dependent upon the employment of such a fixative as faithfully preserved differential structural characteristics.

The material embraced in this investigation, selected from a large stock supply, included embryos from the second to the thirty-second day of incubation, distributed, preserved, and stained as follows:

No. of series.	No. of days of incubation.	Fixing fluid employed.	Stains employed.
Gut open behind cephalic tip of pronephros. 2	Germ cells segregated into two bilateral entodermal cords. 2; 5 somites; 2 mm. length.	Helly......	Iron-hematoxylin and Giemsa.
2	3; 10 somites; 4 mm. length	...Do......	Do.
3	4; 5 mm. length..	...Do......	Do.
1	5; 6 mm. length (germ-cells migrating into visceral mesoderm).	...Do......	Do.
1	6; 6.5 mm. length (migration to sexual gland begun).	...Do......	Giemsa.
1	7; 7 mm. length (open hind-gut beginning to close).	...Do......	Iron-hematoxylin.
3	8; 8 mm. length...............	...Do......	Iron-hematoxylin, and hematoxylin-eosin.
1	9; 9 mm. length.	...Do......	Giemsa.
1	10 (with sharp head and tail bend; 5 mm. greatest length).......	...Do......	Giemsa.
1	11.........................	Flemming..	Iron-hematoxylin.
1	12; 9 mm. greatest length.......	Helly......	Do.
2	13.........................	...Do......	Do.
2	16.........................	...Do......	Iron-hematoxylin, Giemsa, and hematoxylin-eosin.
1	22.........................	...Do......	Delafield's hematoxylin; embryos stained in toto.
1	25.........................	...Do......	Do.
1	25.........................	Flemming..	Iron-hematoxylin.
1	32.........................	Helly......	Delafield's hematoxylin.
25	The sections were cut at from 5 to 10 microns.		

This material was collected on Loggerhead Key, Tortugas, Florida, in the summer of 1914, while at the Laboratory of the Department of Marine Biology of the Carnegie Institution of Washington.

Three nests of eggs were available for the earlier parts of the series. Two additional nests contributed to the later parts of the series. The difficulties of removing the early blastoderm from the yolk were not successfully overcome until the time had passed for preserving stages younger than the end of the second day (5-somite stage). However, this stage shows clearly the origin of the germ-cells from among the yolk-sac entoderm, and the beginning of their segregation into two bilateral cords near the lateral margin of the area pellucida in the caudal half of the blastoderm; and it corresponds closely with von Berenberg-Gossler's illustration (fig. 1) of his youngest lizard embryo (5-somite stage).

Two chief reasons led to the selection of the loggerhead-turtle embryo for this investigation: (1) the possibility of obtaining a large and closely graded series of stages; the number of eggs per nest is near 100; development is comparatively slow, the incubation period being about 8 weeks; (2) I had already familiarised myself in previous works with the embryonic blood-cells of certain *Chelonia* (1913) and also with the male germ-cells in a study of the spermatogenesis of several species of turtles (1914).

The object in using Helly's fluid as a fixative almost exclusively was to preserve the cytoplasmic granules, both albuminoid and lipoid, that is, blood-cell granules and mitochondria, for subsequent differential staining with the Giemsa stain and with iron hematoxylin. Parallel series were prepared with these two stains, with the expectation of facilitating the differentiation between hemoblasts and gametoblasts. This technic was very serviceable, but did not yield quite the fine uniform results hoped for. As concerns the hemoblasts and granular blood-cells (eosinophiles) nothing better could have been desired; but the cytoplasmic contents of the primordial germ-cells were only indifferently preserved. The fixation period should probably have been prolonged for 3 or 4 days beyond the usual 24 hours, especially in the case of the older embryos. Only very rarely were mitochondria and yolk-globules preserved in the later stages. Especially in the germ-cells were the yolk granules dissolved, leaving thus a greatly vacuolated and distorted cytoplasm. But the Helly's fluid and the subsequent treatment involved in the paraffin technic had a very variable effect upon the yolk-granules at different stages. The same type of cell might contain many or only a few, or no yolk-granules. In the latter case the yolk-content had been entirely dissolved. Germ-cells of the 2-day stage of incubation showed many yolk-spherules; by the fourth day no germ-cells contained yolk after this fixation. Obviously the later steps in yolk metabolism were more susceptible after Helly's fixation to the solvent action of the alcohols and oils used in the paraffin technic. The young germ-cells evidently contain less readily soluble yolk than other cells, with the exception of the entodermal cell of the area opaca. In tissues fixed with Helly's fluid and stained with iron-hematoxylin the germ-cells in early stages therefore stand out clearly from among the entoderm-cells of the area pellucida, and accordingly this technic proved to be very favorable for the study of this early, most important, stage.

Some embryos of the eleventh and the twenty-fifth day of incubation were fortunately preserved in Flemming's fluid. It so happens that the 11-day stage of the incubation period is crucial from the viewpoint of the greatest abundance of primordial germ-cells in the area including the closed hind-gut, the mesentery, and the primitive gonad. In these cells mitochondria as well as yolk-granules are well preserved; and so also are the granules of the confusing large blood granulocytes. The germ-cells in the gonads of the 25-day embryo are practically identical with those of the 11-day embryo in form and size and in regard to cytoplasmic content. Comparative study of the 11-day embryo fixed and stained in the three different ways—(1) Flemming's fluid and iron-hematoxylin stain; (2) Helly and iron-hematoxylin; (3) Helly and Giemsa—revealed admirably the diagnostic marks between the germ-cells and cells with which they might be confused, *i. e.*, blood granulocytes, yolk-laden hemoblasts, and yolk-laden entodermal cells. These marks once established at this stage, earlier stages could be much more readily identified in the Helly-Giemsa or Helly-iron-hematoxylin material than in the Flemming material, since the most confusing and obscuring features were here largely eliminated, namely, the very abundant yolk globules and granules.

DESCRIPTIVE.

11-DAY EMBRYO: FLEMMING FIXATION, IRON-HEMATOXYLIN STAIN.

In view of what was said above it seems clear that the best way of approach to the problem was through the 11-day embryo preserved in Flemming's fluid and stained with iron-hematoxylin. The next step involved a comparative study of embryos of this same stage fixed with Helly's fluid and stained respectively with the iron-hematoxylin and the Giemsa mixtures. Thus the criteria for identifying the primordial germ-cell were established and the previous and later history could then be traced with comparative ease.

This stage corresponds closely with von Berenberg-Gossler's illustration (fig. 9) of a lizard embryo. Primordial germ-cells are present among the entoderm-cells of the closed hind-gut and in the surrounding mesenchyma; in the mesentery, both in the mesenchyma and its mesothelial covering; and in the primitive genital gland, both among the peritoneal cells and in the subjacent mesenchyma (see fig. 2, plate 6). Figure 1, plate 1, is a composite drawing including the several types of cells with which we are now concerned, all from the same immediate region of the mesentery. We may compare first cells *a* and *b*, a typical germ-cell and a typical eosinophil granulocyte, both in the mesenchyma of the mesentery. Cell-type *b* may appear at any stage after the second day of incubation in any part of the yolk-sac or embryonal mesenchyma, including the blood-vessels. At first sight it would seem to correspond to what Swift described as a primordial germ-cell circulating in the blood-vessels of the young chick embryo. It contains an attraction sphere (*i*), a spherical deep-staining chromatic nucleus, and abundant small spherical granules (apparently comparable to the granular mitochondria of germ-cells); but the resemblance is only very superficial and no confusion need arise.

Cell *a* is a typical primordial germ-cell. Ordinarily, in the inactive condition, it has an oval form, rather than the more nearly spherical form of the granulocyte in the same condition. Moreover, it is nearly twice the size of the largest granulocyte. Its nucleus is vesicular, contains a very characteristic finely granular karyoplasm, and stains only very lightly in basic dyes. The radial disposition of the rows of oxyphilic chromatin granules is striking. There is present usually only one large, very chromatic, spherical or oval nucleus; and one (or sometimes several) much smaller chromatic accessory nucleoli; and usually one (sometimes several) pale plastin nucleoli. The cytoplasm is more or less crowded with larger and smaller yolk spherules, staining more or less deeply. It contains also abundant granular, bacillary, beaded, and filiform mitochondria (fig. 2*a*, plate 1).

The granules of the blood granulocyte, on the contrary, are quite uniform in size and in their deep-staining quality. The nucleus of the granulocyte also is relatively smaller, contains a relatively coarse chromatic net-work, and stains much more deeply as a whole in basic dyes. In anticipation of what follows it may here be stated to advantage that primordial germ-cells of earlier stages, including the 2-day stage, are of approximately the size of those of the 11-day stage, only occasionally slightly smaller. The germ-cells are therefore always larger than the largest granulocyte (plate 1, figs. 1*b* and 2*b*), the largest hemoblast (plate 1, fig. 1*h*), and the largest erythroblast (plate 1, fig. 5). The nucleus of the germ-cell also is quite different from that of any of the cells in close proximity to which it may be found—granulocyte (*b*), mesothelium (*M*), mesenchyma, hemoblast (*h*), erythroblast (fig. 5), and erythrocyte (plate 1, fig. 1*e*). It resembles closely only the young entodermal cell, especially in respect of its nucleus, from which cell the germ-cell can, however, be distinguished by criteria which will be given below. Moreover, the granulocyte may assume most varied shapes, at different stages of amœboid progression; the germ-cell, while also capable of amœboid movement, becomes less irregularly modified, extreme forms being represented in figure 3, *aa*, *ab*, *ac*, plate 1.

In the Helly fixed tissue, stained with Giemsa, the granules of the blood granulocytes stain either blue or red, depending upon the phase of development of the cells. The majority of the cells at this stage contain red-staining granules; a few contain only unripe blue-staining granules; and a certain number have mixed granules, including all phases of the ripening process of an eosinophil granule. The nucleus also stains a deep blue. The primordial germ-cell of this tissue, on the contrary, has a light bluish-pink-staining nucleus, with a deep purple nucleolus. The cytoplasm of the germ-cell is vacuolated, the vacuoles being the negative of the dissolved yolk-globules; and it has a coarsely reticular character in consequence, and stains only faintly pink.

Already, from a study of only this stage of development, much information can be gained concerning the fact, route, and method of the migration of the germ-cells from the gut entoderm to the peritoneal epithelium of the gonad. It may be stated here that at no stages studied was any primordial germ-cell, with two exceptions, ever seen in a blood-vessel. The blood-channels in this form evidently do not greatly assist in the transfer of the germ-cells to the sex-gland, contrary to what Swift has demonstrated for the chick; nor has such a route been advocated for any other form except duck (von Berenberg-Gossler); but that the cells do migrate is indicated by the different locations occupied, progressively nearer the genital ridge, corresponding with successively later developmental stages, and that the migration is the result of an inherent amœboid capacity is shown by the various shapes assumed by these cells (figs. 3*aa*, 3*ab*, plate 1); and the path of migration is quite clearly indicated by the pointed condition of the forward end, combined with the altered (flattened and distorted) condition of the cells lying directly in the path of progression. For instance, the primordial germ-cells among the entodermal cells of the closed hind-gut in the 11-day embryo are surrounded by entodermal cells which have become crowded, flattened, and distorted on either side as if through pressure by a cell which enlarged and assumed a spherical shape *in situ*. The similarity in structure and staining reaction between the germ-cells and the entodermal cells, a similarity which becomes progressively closer as earlier stages are approached, seems to speak in favor of an entodermal origin of the germ-cells or a close genetic relationship between entoderm and germ-cells. When the germ-cell lies near the basement membrane of the primitive gut entoderm, the cellular distortion is such as would be caused by a cell migrating from the entoderm into the surrounding mesenchyma. Occasionally a germ-cell can be seen partially within the entoderm and partially within the mesenchyma, an observation which proves a migratory process; and the manner of the distortion of the mesenchymal cells shows that this migration is in a peripheral direction.

Within the mesenchyma of the gut and the mesentery the distortion and flattening of the cells are generally in the direction of the root of the mesentery, thus showing a migratory progression in that direction. Occasional cells, judged by this criterion, are moving towards the mesothelial layer of the mesentery (figs. 3*aa*, 3*ab*, plate 1). Occasional cells are also found among the cells of the peritoneal epithelium of the genital ridge. In the region of the angle between the root of the mesentery and the gonad, and again within the mesenchyma of the sexual gland, the shape of cell and the distortion of the adjacent mesenchymal nuclei are such as to indicate a ventro-medial progression to the surface of the gonad, where the germ-cells come to rest among the peritoneal cells (fig. 2, plate 1). Figure 3*ac*, plate 1, shows a germ-cell similarly located, but almost completely filled with a huge yolk-mass.

The germ-cells are apparently most abundant at about the 11-day stage. This increase is probably merely apparent, due to the segregation of the cells within relatively more restricted and conspicuous limits, viz, gut and mesentery. During earlier stages (to the seventh day), when the gut is still entirely open over the yolk, their distribution is scattered over a wider area; also in later stages, when they have taken their definite position within the enlarged gonad. The number of germ-cells in the 11-day embryo, all situated behind the level of the cephalic tip of the pronephric duct, is approximately 400. Only an occasional cell may be seen at this stage in mitosis. Similarly, during the later stages mitosis is a very rare occurrence. In the stages of 2 to 4 days a few of the germ-cells are in process of division among the entodermal cells. Cells in division are apparently of smaller size and contain relatively little yolk.

10-DAY EMBRYO; HELLY FIXATION, GIEMSA STAIN.

In a 10-day embryo the primordial germ-cells are found in about the same locations; they are slightly smaller, but the smaller size is only apparent, due to a greater cytoplasmic shrinkage in this fluid (fig. 4*a*, plate 1), in part also very probably to the loss of the large yolk-content. Moreover, the Flemming fluid may have caused a slight swelling. The cytoplasm is here vacuolated and coarsely reticular, the result of the solution of yolk-granules. In figure 4, *a*, *b*, *c*, *d*, and *e*, plate 1, are shown primordial germ-cells from the gut, mesentery, and peritoneal epithelium of this stage. For comparison a blood-granulocyte (*f*) is added.

12-DAY EMBRYO; HELLY FIXATION, IRON-HEMATOXYLIN STAIN.

At the twelfth day the germ-cells are still scattered among the entodermal cells of the hind-gut, in the mesentery, and in the genital ridge. Figure 6*a*, plate 1, is taken from among the entodermal cells of the gut; *b*, from among the mesothelial cells of the genital ridge; *c*, from the mesenchyma of the mesentery. The adjacent mesenchymal cells have become changed by the pressure of the migrating cell into flattened encapsulating elements.

The accompanying chart shows the distribution of the germ-cells at this stage, and their condition in different locations. The total number of cells counted is 352, about equally divided between the two gonads (118 in left, 127 in right). A few cells are in mitosis, mostly situated among the entodermal cells. Some are in process of degeneration; such are found chiefly in the mesenchyma of the gut. One cell is situated in what appears to be a vascular space, close to the aorta; another is in process of degeneration within a blood-vessel; seven occur in the periaortic mesenchyma; and four among the neuroblasts of the developing periaortic sympathetic ganglia (fig. 2, plate 4).[1]

---

[1] The photomicrographs were made by Mr. Wm. S. Dunn, Cornell University Medical School New York.

The periaortic germ-cells migrated dorsally (perhaps only temporarily) instead of laterally across the cœlomic angle, some apparently finding their way into blood-channels. The germ-cells within the gonads are about equally divided between the mesothelium and the subjacent mesenchyma. The mesothelium consists of only a single layer of prismatic cells, greatly flattened where they overlie a germ-cell.

Chart showing the distribution of the germ-cells in a 12-day embryo.

The cells occur from the level of the open gut to that of the end of the closed hind-gut, from slides 11 to 16, a distance of 3 mm. Slides 11 and 12, a distance of 0.8 mm., include the open portion of the hind-gut.

	Gut.		Mesentery.		Genital ridge.		General body mesenchyma.	Total.
	Entoderm.	Mesenchyma.	Mesothelium.	Mesenchyma.	Left gonad.	Right gonad.		
Slide 11..	....	....	1 near root......	1 near root..	....	1	....	3
Slide 12; open gut figure 1, plate 6.	1 in mitosis; 2 near periphery.	7 dorsally.......	1 at prophase of mitosis; 3 at root; 1 in lower third; 1 near gut end.	1 near root; 2 at gut end.	3	2	....	24
Slide 13; closed gut, figure 2, plate 6.	2 in mitosis; 5 dorsally.	2 ventrally (degenerating); 3 laterally (degenerating); 3 dorsally; 2 laterally.	....	2 at gut end; 2 at root end.	9	1	....	31
Slide 14..	1 in mitosis; 2 degenerating; 7 normal.	5 degenerating; 5 dorsally; 6 laterally; 2 ventrally.	1 near middle point; 1 near root.	1 near root end; 1 near gut end; 1 near middle point.	30	32	....	95
Slide 15; figure 3, plate 6.	3 near periphery.	1 laterally.......	6 near root end; 2 near gut end; 1 near middle point.	2 near root; 2 near gut.	70	82	....	169
Slide 16..	1 dorsally...	....	....	1 near gut end.	6	9	1 degenerating in blood-vessel; 7 periaortic; 1 in vascular space; 4 among periaortic sympathetic ganglion cells. Figure 2, plate 4.	30
					118	127		352

OLDER EMBRYOS; FROM 13 TO 32 DAYS.

The 13-day embryo is essentially like the one of 12 days (figs. 1, 2, and 3, plate 6), except that relatively fewer germ-cells remain in the gut and more are found within the gonads. In the 16-day embryo (11 mm. in length, from cephalic to caudal bend) the germ-cells have practically all migrated into the gonads, where they come to rest, both among the mesothelial cells and in the subjacent mesenchyma. In the stouter mid-portion of the genital ridge (future gonad), where the mesenchyma is more extensive, the majority of the primordial germ-cells lie subepithelially; in the cephalic and caudal extremities, on the contrary, where the mesenchyma is sparse, the majority of the germ-cells lie in the peritoneal epithelium, having pressed the columnar cells aside laterally and caused great flattening of the overlying cells. Only an occasional cell is found within the gut and the mesentery. In three slides, including 60 sections, through the hind-gut, three germ-cells were seen in the mesentery (one at the gut end and two in the mid-portion, all apparently in healthy condition), and one in the mesenchyma of the gut. Many of the germ-cells within the gonads at this stage have a coarsely granular nuclear reticulum (fig. 7, plate 2), the granules staining deeply, in contrast to the pale nucleus with very delicate reticulum of the germ-cells of earlier stages.

In figure 7, plate 2, is illustrated a primordial germ-cell from the epithelial layer of the gonad of a 22-day embryo (12 mm. in length). An occasional germ-cell still occurs also in the gut and in the mesentery. The germ-cells of this stage have the same structure and approximately the same size as those from the 10 to 16 day periods, but relatively more cells contain coarsely granular nuclei than in the 16-day stage. The germ-cells of the 32-day stage are no larger than those of the earlier periods, as shown in figure 9, plate 2, of a cell taken from the subepithelial mesenchyma of the gonad. In figure 8, plate 2, is illustrated a germ-cell from the mesenchyma of the mesentery of a 25-day embryo. This particular cell, in common with many others, appears to be encapsulated by flattened mesenchymal-cells. The flattening of the mesenchymal cells is the result of pressure produced by the migrating germ-cell in the direction of the root of the mesentery. It may be emphasized that the germ-cell has undergone no growth or other striking structural alteration between the tenth and thirty-second day of incubation. (Compare figs. 4 and 6, plate 1, with fig. 9, plate 2.) By the sixteenth day all the germ-cells, except a few strays, have left the gut and mesentery and are located within the sexual glands. Also at the 25-day stage an occasional cell may still lag behind in the gut and mesentery; and even in the 32-day embryo several cells were seen in the mesenchyma of the gut, though these were apparently in process of degeneration. The migration process appears to be at its height from the seventh to the sixteenth days; but certain stray cells, mostly degenerating, still persist extra-regionally at the thirty-second day.

In a 25-day embryo, fixed in Flemming's fluid and stained with iron-hematoxylin, where the germ-cells could be more readily detected because their content of yolk-globules was perfectly preserved, 3 germ-cells were seen (in 4 consecutive slides, including 80 sections) in the root of the mesentery—one (degenerating) near the gut end, one in the mid-portion, and one (degenerating) in the mesenchyma of the hind-gut ventrally. The germ-cells of the embryo, in contrast to all other cells (except liver-cells), still showed abundant yolk-granules and some mitochondria.

The migration period is obviously not sharply limited, but extends in some degree through half the incubation period. Some germ-cells apparently lag hopelessly behind and degenerate, especially in the gut. The initial conspicuous marks of degeneration relate to stages in the disappearance of the nucleus either by solution or karyorrhexis.

YOUNGER EMBRYOS; FROM 9 TO 6 DAYS.

The 9-day embryo (figs. 1 and 2, plate 5) differs from the 10-day embryo only in the relative abundance of the germ-cells at different levels of the migration route; the germ-cells are relatlvely less abundant in the higher levels and in the gonads.

In figure 10*a*, plate 2, is illustrated a germ-cell of an 8-day embryo taken from the splanchnic mesoderm at about the mid-region of the area pellucida. It appears to lie in a lacuna, the product of shrinkage; *b* is taken from the mesentery and is closely enveloped by mesenchymal cells. The latter germ-cell has a diameter of only about 13 microns; *a*, about 16 microns. For comparison, two hemoblasts are included, *c* and *d*. The germ-cells at this stage are most abundant in the mesentery and in the median portion of the splanchnopleure.

In the 7-day embryo the hind-gut is open throughout—that is, beyond the anterior tip of the pronephros. The mesentery is relatively very long caudally. Some germ-cells are found among the entodermal cells medially, but the majority are in the long mesentery, widely scattered, both among the mesenchymal and the mesothelial cells, and in the medial extremity of the visceral plate of mesoderm. A few cells are already found within the genital ridge and a few remain scattered among the entodermal cells of the open gut. In figure 12, plate 2, is illustrated a large germ-cell from among the entodermal cells of the hind-gut, at the point of initial closure. The cytoplasm contains enormous peripheral vacuoles and is much shrunken, due to the dissolution of the yolk-masses of the cell. Figure 11, plate 2, shows a younger germ-cell among the entoderm-cells of the area pellucida, near the mid-line. The difference in size of these two cells (16 microns and 20 microns) might be interpreted to indicate a considerable growth of the germ-cells at about the 7-day stage, but it more probably indicates merely a normal size variation, since large germ-cells appear already at the 3-day stage (10 somites) (figs. 13, 14, and 15, plate 2). Swift (1914) records a variation in diameter of the primordial germ-cells in the chick embryo of from 14 to 22 microns, the average being about 16 microns. In this embryo a few germ-cells among the entodermal cells of the open gut and in the mesothelium of the mesentery are in mitosis.

The body of the 6-day embryo lies almost flat upon the yolk; the gut is open behind the cephalic extremity of the pronephros. Only at this point is there a mesentery, and it is very short and contains no germ-cells. The germ-cells occur in both the entoderm and mesoderm of the open gut, the majority lying scattered in the mesoderm medially.

YOUNGEST EMBRYOS; FROM 2 TO 5 DAYS.

Towards the end of the second day of incubation, when 5 somites are formed, the primordial germ-cells are just becoming segregated within the entoderm of the area pelllucida. The cells now occur in two bilateral cords, more or less oval in transection. These cords extend from just in front of the primitive pit (neurenteric canal) to the caudal end of the primitive streak; they lie laterally in the area pellucida. The germ-cells are not yet as closely aggregated as during the following day. Cells can still be found in process of migration from the yolk-sac entoderm of the area opaca to the entoderm of the area pellucida.

At the 3-day stage (10 somites) the cords of primordial germ-cells are more closely segregated and have apparently moved slightly more medially (fig. 1, plate 3). They now extend from the sixth somite to the caudal extremity of the primitive streak. These cords do not have a uniform diameter throughout, but are more or less interrupted, suggesting a segmental condition, recalling the "gonotome theory" of Rückert.

Figure 15, plate 2, shows a germ-cell from among the yolk-laden entodermal cells of the area opaca of the 3-day stage; an entoderm-cell nucleus is added at the right (*a*) to show the similarity of nuclear condition between the germ and entodermal cells. Figure 14, plate 2, shows a germ-cell taken from the area pellucida. This cell has a diameter of 16.5 microns. Figure 13, plate 2, shows a similar cell in process of migration from among the entodermal cells of the area pellucida into the overlying visceral layer of the lateral mesoderm. Closely similar conditions appear also at the 2-day stage.

The 5-day stage (fig. 2, plate 3, and fig. 1, plate 4) represents a crucial epoch in the early history of the primordial germ-cells. The gut is open throughout and the embryo lies flat upon the yolk. The entodermal cords of germ-cells have made an intimate linear contact with the visceral plate of the lateral mesoderm (fig. 1, plate 4), and are beginning to pass into the latter and to migrate medially. The 4-day stage shows transition conditions between those described for the 3 and 5 day stages. Meanwhile the angioblast layer of the visceral mesoderm is becoming vascularized, and an occasional cell may become inclosed by, or migrate into, a blood-channel, from which it again migrates into the surrounding mesenchyma or possibly later degenerates somewhere in the blood-vessels.

Between the first and sixth days, then, the scattered primordial germ-cells have become segregated from out of the area opaca into a pair of bilateral cords lying near the lateral border of the area pellucida in approximately the caudal half of the embryonic area (fig. 1, plate 3), whence they pass at about the fourth to fifth days into the visceral plate of the lateral mesoderm (fig 1, plate 4), and thence medially towards and into the forming mesentery of the just-closing hind-gut (fig. 1, plate 5). Certain stray germ-cells may persist in any portion of the hind-gut and its mesentery until about the 32-day stage, at which time the final remnant largely degenerates, a few possibly persisting and perhaps furnishing foci for future neoplastic growths.

The very close resemblance between the nuclei of the entodermal cells of the area opaca and the included germ-cells seems to indicate a very close genetic relationship; and their cytomorphic features disclose an equally undifferentiated condition. The only striking difference between these cells is one of shape; the germ-cell is oval, the entoderm-cell very irregular and apparently in syncytial relationship with other entodermal cells.

LITERATURE.

GERM-CELL ORIGIN IN INVERTEBRATES.

The clearest and most striking case of early germ-cell differentiation among the invertebrates is that of *Ascaris megalocephala*, in which Boveri (1892) traced the germ-cells through the cleavage back to the 2-cell stage. The conspicuous differential feature of this cell at the 2-cell stage (and to the sixth cleavage) is the maintenance at mitosis on the part of one of its daughter-cells of the integrity of the chromosomes in contrast with the soma-cell, both of whose daughter-cells suffer a diminution of the chromatin at each division. The germ-cells become segregated at the 32-cell stage of cleavage, after which they produce only similar germ-cells at each division.

Previously Balbiani (1885) had succeeded in tracing the germ-cells ("pole-cells") of the dipter *Chironomus* to an early stage of differentiation in the segmenting egg. "Pole-cells" were first reported by Robin in 1862 for certain other Diptera, and subsequently (1863) also by Weismann for *Chironomus*. Hasper has quite recently (1911) identified one of the first four cleavage-cells as the progenitor of all the germ-cells in *Chironomus*.

A similar history was traced by Häcker for the germ-cells in *Cyclops;* here the germ-cell progenitor is recognizable at the 32-cell stage.

Hegner (1909, 1914, 1915) has worked out very completely the early germ-cell path ("Keimbahn") in certain Diptera and in chrysomelid beetles, where the diagnostic marks are a polar differential granular cytoplasmic content, the "pole-disk," constituting a germ-cell determinant. In the dipter *Minastor*, Hegner (1914) describes a segregation of the germ-cells at the 8-cell stage, in a number of other cases at the 32-cell stage.

The literature touching the germinal path ("Keimbahn") in invertebrates is fully and very ably reviewed by Hegner in the Journal of Morphology (vol. 20, p. 231; vol. 25, p. 375; and vol. 26, p. 495). The reader is referred to these papers for bibliographic lists.

GERM-CELL ORIGIN IN VERTEBRATES.

The detailed and critical study of the origin of the germ-cells in vertebrates may be said to have begun with the observations of Hoffman (1893) on certain birds (including *Gallinula chloropus*, *Sterna paradisea*, and *Hæmotopus ostralegus*), which disclosed primordial germ-cells in the entoderm and splanchnic mesoderm far removed from the site of the future gonads, and before a "germinal epithelium" had developed (23-somite stage).

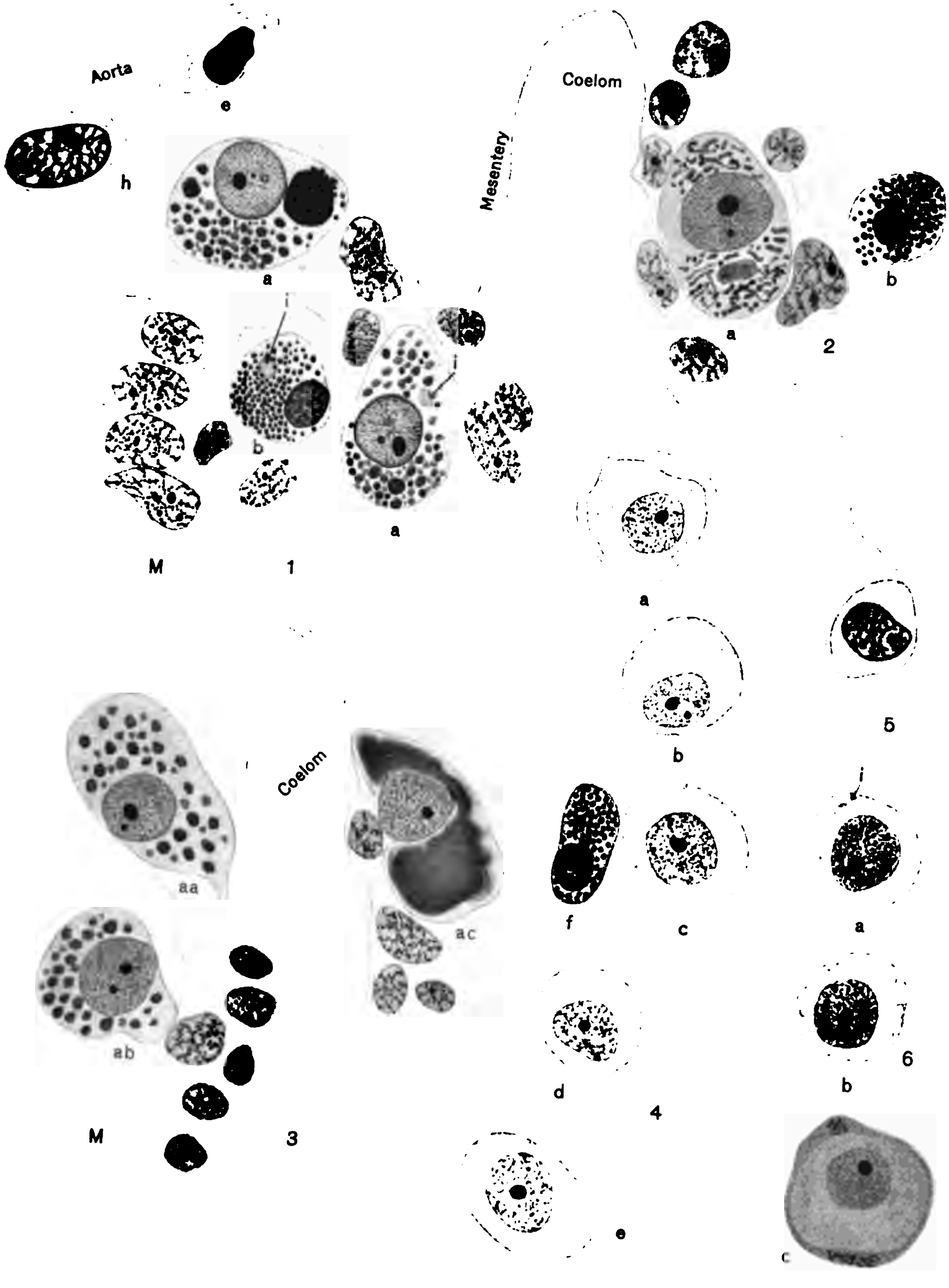
The work of Eigenmann (1892, 1897) marked an epoch in this line of investigation. He clearly demonstrated the extra-regional origin of primordial germ-cells in the viviparous teleost *Cymatogaster* and showed that they closely approximated the size and appearance of the fifth-cleavage blastomeres, on the basis of which he argued that the germ-cells were segregated at the fifth-cleavage stage. An extra-regional entodermal origin of primordial germ-cells has been described also by Dodds (1910) in the teleost *Lophius*.

In 1904 D'Hollander described the origin of the germ-cells (oögonia) in the chick embryo of the tenth day from epithelial buds of the peritoneal epithelium, in conformity with the earlier views of Waldeyer. This discrepant observation is now elucidated by the recent works of Swift (1915, 1916), which show that such buds do indeed occur, but that they *include*, but do not *originate*, primordial germ-cells. The latter have an extra-regional origin.

Beard in 1904 described the extra-regional origin of primordial germ-cells also in the skate *Raja batis*. These cells are said to arise in the anterior portion of the embryonic shield and to migrate along a very definite route to the gut entoderm, thence through the splanchnic mesoderm to the future gonad. A very similar later history has been described by Woods (1902) also for the dog-fish, *Squalus acanthias*.

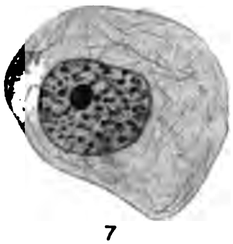
Beard and Eigenmann agree that the germ-cells do not divide for a long period in the early development of the embryo, that the original number remains constant except for a slight diminution due to degeneration, and that a few may become stranded in the migratory process in locations outside of the genital glands.

The most detailed and satisfactory study of the germ-cells in the chick has been made by Swift (1914, 1915, 1916) with the aid of mitochondrial technics. He describes the origin of the primordial germ-cells in a crescentic antero-lateral area from germ-wall entoderm

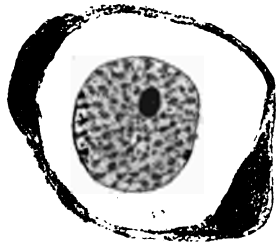


GERM CELLS IN CARETTA

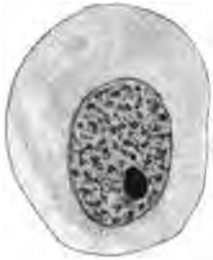
A.Hoen & Co. Lith.



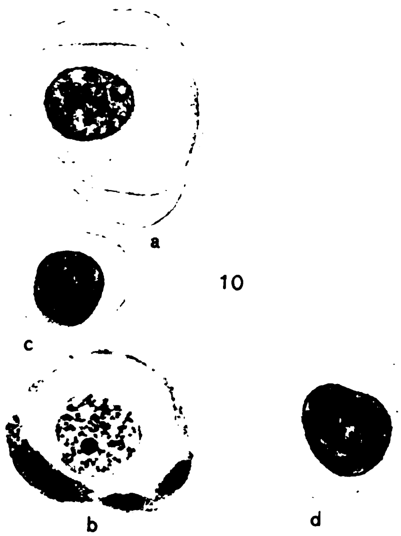
7



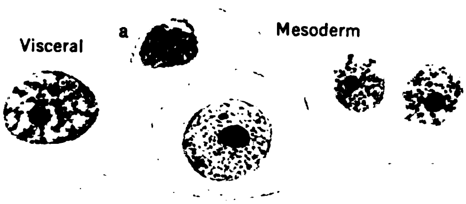
8



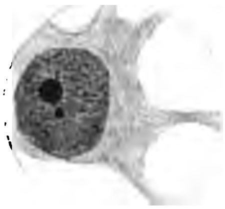
9



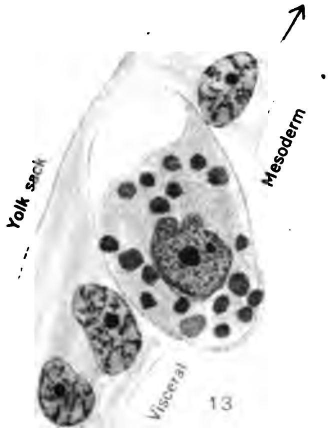
10



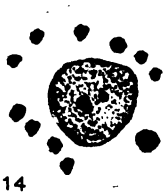
11



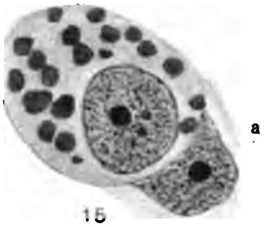
12



13



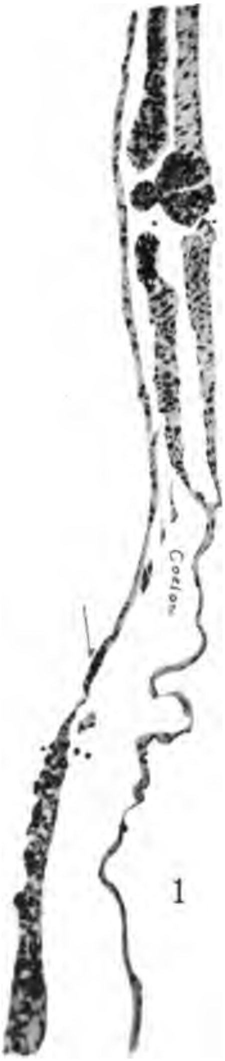
14



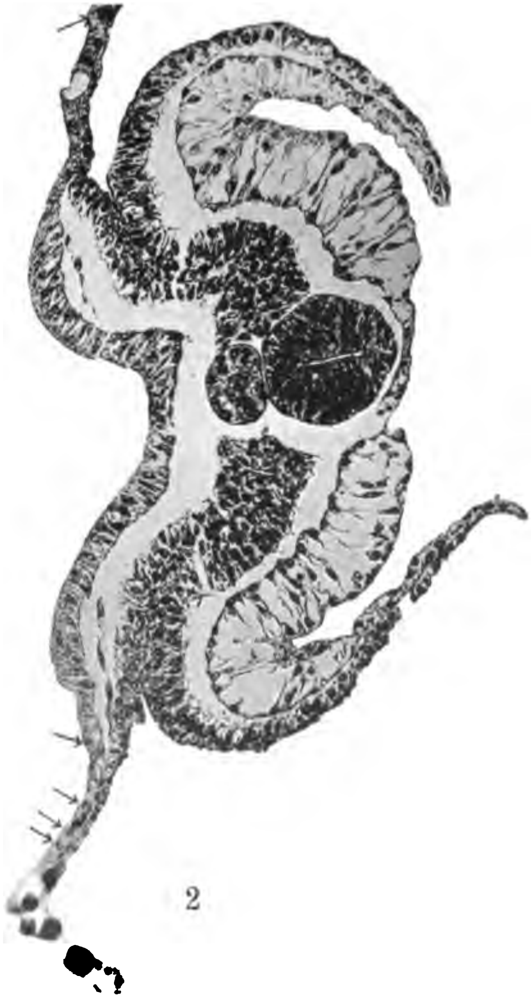
15

A Hoen & Co. Lith

GERM CELLS IN CARETTA



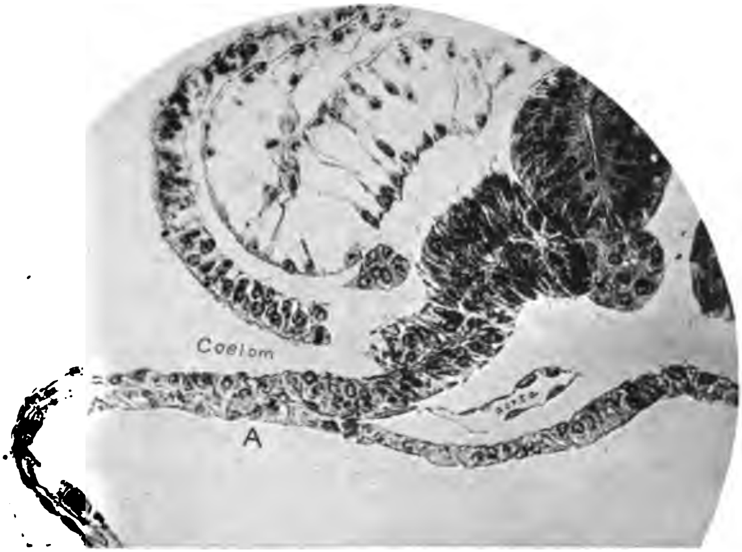
1



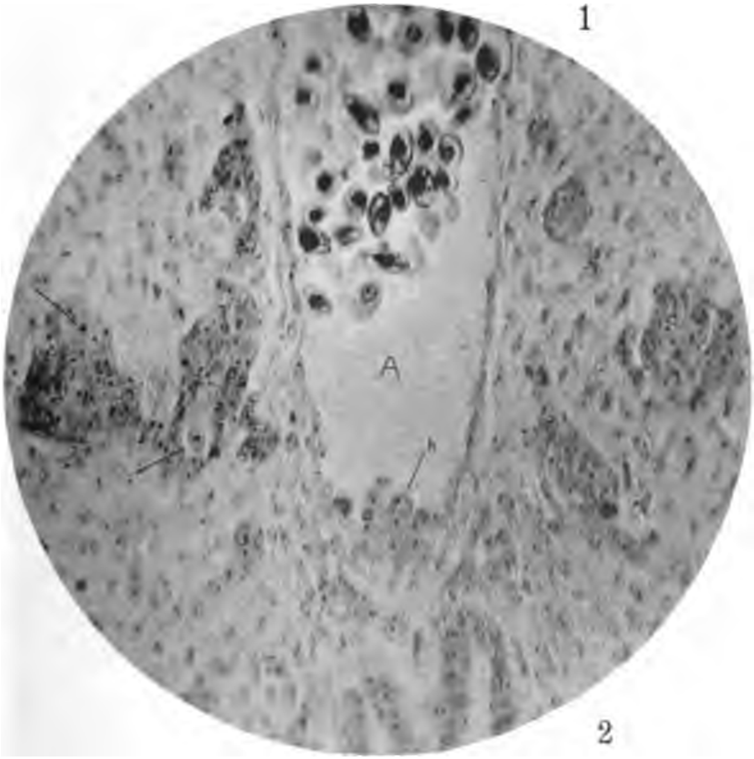
2

1. Transverse section of a 3-day embryo at level of tenth somite. The arrow points to the cord of primordial germ-cells, extending from the sixth somite to caudal extremity of primitive streak, at the lateral border of the area pellucida. Helly fixation; iron-hematoxylin stain. Photo. x 40

2. Transverse section of a 5-day embryo at level near cephalic extremity of the entodermal cords of primordial germ-cells. The two bilateral cords are more or less interrupted or segmented. This section passes through an intersegmental region. Certain germ-cells are also scattered more medially among the entodermal cells of the area pellucida. Arrows point to germ-cells. Helly fixation; iron-hematoxylin stain. Photo x 66

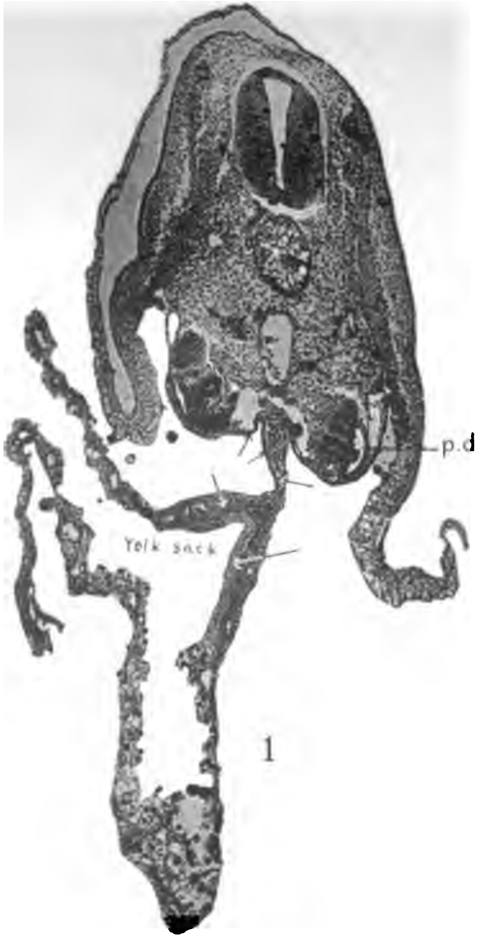


1

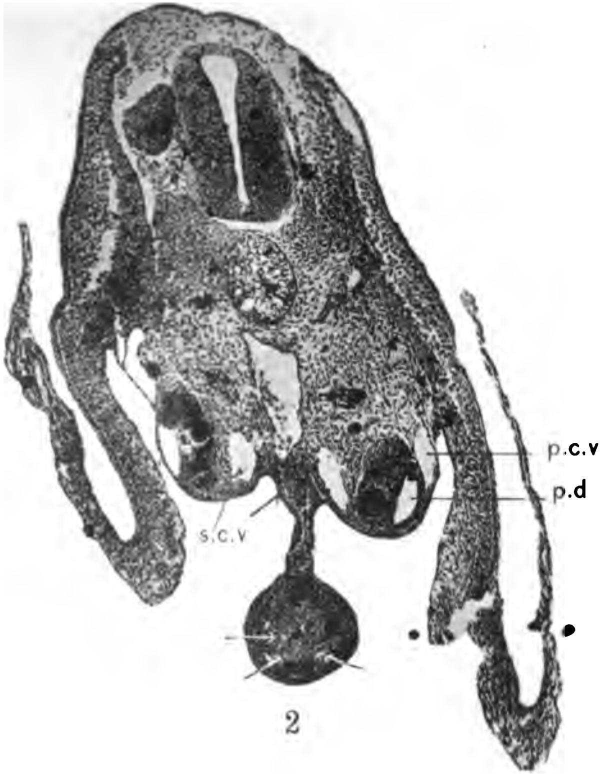


2

1. Transverse section of same 5-day embryo at a level 300 microns forward of section fig. 2, plate 3, through the cord of segregated germ-cells (A) at left. The cord has made a linear connection with the overlying visceral mesoderm and the germ-cells are migrating into it and moving medially. Photo. x 100.
2. Section through abdominal aorta and adjacent masses of sympathetic neuroblasts, 150 microns behind section fig. 3, plate 6. The arrows point to the primordial germ-cells among the neuroblasts. (A) aorta filled with blood dorsally; (*h*), hemoblasts separating from endothelium ventrally. Photo. x 400. (Helly fixation; iron-hematoxylin stain.)



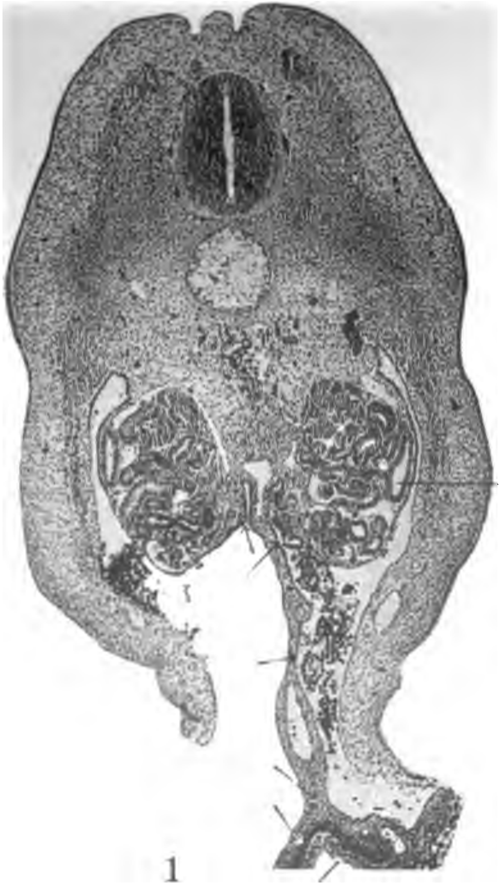
1

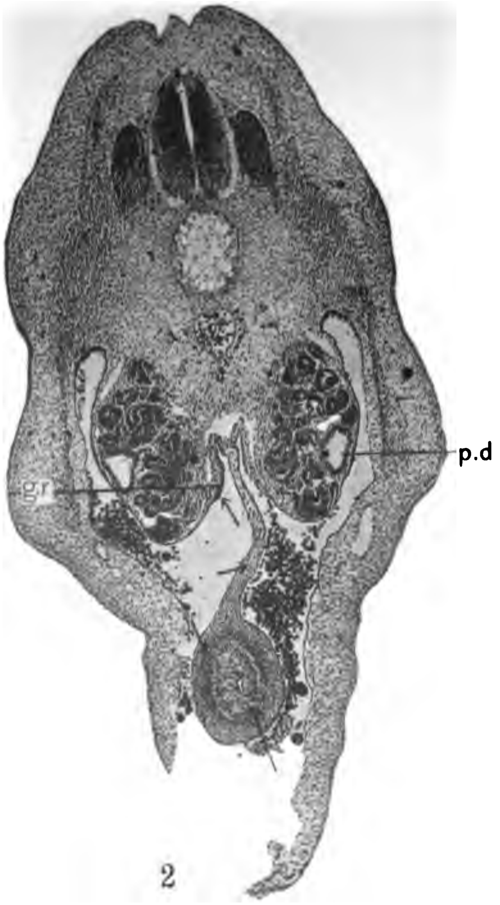


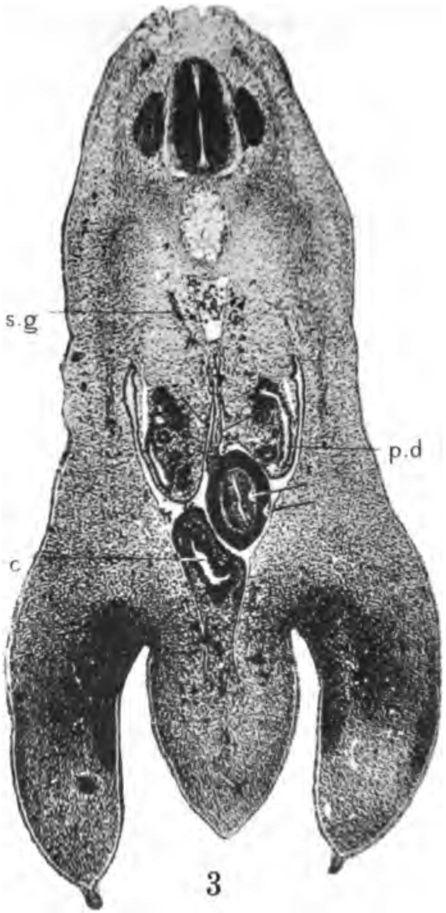
2

1. Transverse section of 9-day embryo and attached yolk-sac near level of cephalic extremity of mesonephros. The arrows point to different locations of the germ-cells (illustrated in figs. 1, 2, 3, and 4, Plate 1). Helly fixation, Giemsa stain. Photo. x 40. The extraneous material consists of broken yolk-globules.

2. Transverse section of same 9-day embryo at level of closed hind-gut, 250 microns caudad of preceding section, fig. 1. Arrows point to germ-cells. (*p. c. v.*), postcardinal vein; (*s. c. v.*), subcardinal vein; (*p. d.*), Wolffian duct. Photo. x 40. The dark irregular masses in this section consist of yolk-globules broken and scattered in sectioning.







1. Photomicrograph of a transverse section of 12-day embryo at level of anteriorly open hind-gut. The arrows point to different locations of primordial germ-cells (illustrated in figure 6, plate 1). (*p. d.*) Wolffian duct. x 47. The cœlomic cavity contains blood-cells and yolk-globules.
2. Transverse section at level of closed hind-gut of same 12-day embryo, 300 microns behind section, fig. 1. The arrows point to germ-cells. (*p. d.*), Wolffian duct; (*g.r.*), genital ridge. Photo. x 47. The cœlom contains blood and yolk globules.
3. Transverse section of same 12-day embryo, 550 microns caudad of fig. 2, at level of cloaca (*c*). (*s. g.*), sympathetic ganglion cells, among which a few primordial germ-cells have migrated. Arrows point to the locations of germ-cells. Photo. x 40.

at the margin of the area pellucida, during the primitive-streak stage and until 3 somites are formed (23-hour stage). The cells by amœboid activity enter the blood-spaces of the mesoderm, which subsequently grows into this portion of the primitive proamniotic area, and are then carried to all parts of the embryo and the vascular area. The germ-cells remain widely distributed in this way until about the time when the embryo has 20 somites. Numbers of those in vascular channels are said to undergo mitotic division, while some suffer degeneration. The germ-cells remain scattered throughout the blood-vessels until about the 22-somite (44-hour) stage, only a few cells appearing in the splanchnic mesoderm. At the 23 to 25 somite (50-hour) stages the majority of the germ-cells have escaped from the blood-vessels and are now found almost exclusively in the tissue of the splanchnic mesoderm near the cœlomic angle. After the 25-somite stage no germ-cells remain in the blood-vessels. In embryos of from 30 to 33 somites (60 to 72 hours) the primordial germ-cells have migrated to the root of the mesentery and into the cœlomic epithelium on both sides of the cœlomic angle. Here they remain until the formation of the gonad begins, when they gradually pass into that organ and become the oögonia and the spermogonia of the ovary and testes respectively.

Swift describes also a very prominent attraction-sphere, persisting without change from the origin of the germ-cell to its entrance into the gonad, and he uses this mark as the chief character for differentiating between germ-cells and adjacent elements. Such a uniform and conspicuous criterion was lacking in the loggerhead-turtle embryo and could not be employed in the identification of the germ-cells.

Others of the more recent workers with chick embryos include Nussbaum (1901), Rubaschkin (1907), and von Berenberg-Gossler (1912). These investigators agree in finding primordial germ-cells in the entoderm and splanchnic mesoderm lateral to the cœlomic angle in embryos of about 22 somites. In a more recent paper von Berenberg-Gossler (1915) confirms Swift in his observations that primordial germ-cells appear within the vascular channels in chick embryos prior to the 25-somite stage.

Firket (1914) maintains that in birds the majority of the primordial germ-cells disintegrate, though some may possibly come to "maturity" in the wall of the gut; and he views their extra-regional appearance as of only phylogenetic significance, reminiscent of the definitive sex-cells of lower vertebrates.

The work of Allen on the turtle *Chrysemys marginata* (1906, 1907) and on the ganoid fishes *Amia* and *Lepidosteus* (1911) has greatly advanced our knowledge regarding the origin and development of the primordial germ-cells in vertebrates. In these three forms Allen has likewise demonstrated an extra-regional entodermal origin. In *Chrysemys* the germ-cells ("sex-cells," Allen) are described as arising from the entoderm near the lateral margin of the area pellucida at a level beginning about the cephalic extremity of the pronephros and extending on either side to a point just behind the caudal tip of the embryo. The total number of the original quota of germ-cells is said to vary from 302 to 1,744, with an average of about 1,100 (Allen, 1907). No mitoses were observed in these cells until the 10-mm. stage of development. During the migration of the sex-cells many are said to lag hopelessly behind, while others may go entirely astray. Upon an average 47.7 per cent are estimated to finally reach the sex-glands, while the remainder are said to come to rest in the alimentary tract, the mesentery, and the region between the root of the mesentery, the aorta, and the mesonephroi.

It is important to note that Dustin (1910) records a much smaller average number of germ-cells in *Chrysemys* embryos of this stage, the extremes being given as 158 and 415. This number is much closer to my own count (see chart, page 323) for *Caretta*.

In *Lepidosteus* the primordial germ-cells have a very similar origin. They are described as migratory to a high degree. In *Amia* they are said to arise from the entoderm of the roof and margin of the floor of the subgerminal cavity.

The history of the germ-cells in *Chrysemys* is practically identical with that above described for *Caretta*. However, I find no evidence in *Caretta* to support Allen's conclusion that "large numbers" of cells fail to migrate eventually to the gonads, but undergo degeneration in these extra-regional locations. Rarity of actual degenerating cells, or persisting representatives of any kind in later stages, indicates an ultimate migration of at least the vast majority of the extra-regional germ-cells into the genital-glands. Moreover, an occasional germ-cell may be seen dividing during all the earlier stages.

Dustin (1907) describes the origin of the sex-cells ("gonocytes") in a 3 mm. *Triton* larva from the medial portions of the lateral plates of mesoderm in the caudal half of the body. These original sex-cell masses become approximated medially and subsequently fuse to form a longitudinal rod of sex-cells just above the dorsal root of the mesentery. At about the 14 mm. stage the majority of the original sex-cells are said to degenerate and disappear, and a new generation to be differentiated from the peritoneal epithelium of the genital ridge. A similar mode of origin and history of the germ-cells is described for *Rana* and *Bufo*. As regards *Rana pipiens*, Allen (1907) describes a dorso-medial migration of germ-cells from the gut entoderm at the time when the two lateral plates are approximated in the formation of the mesentery. The initial mesodermal origin described by Dustin is accordingly here an illusion.

In the matter of an entodermal origin of the germ-cells in *Rana*, Allen has been confirmed by Kuschakewitsch (1908), and by King

(1908) as respects *Bufo lentiginosus*. Miss King finds no evidence in support of a secondary source of germ-cells from the peritoneal epithelium. Moreover, in *Chrysemys*, Allen (1906) traced the germ-cells to maturity without finding any evidence of a transformation of peritoneal cells into germ-cells. The same is true also of *Caretta* at least to the 32-day stage.

Dustin (1910) has repeated the investigation of Allen on *Chrysemys marginata*, and now confirms Allen's observations regarding the entodermal origin of the extra-regional germ-cells in this form. Dustin still claims, however, that in this form also there is a secondary peritoneal source of origin of germ-cells or "gonocytes." But his illustrations do not seem to support this conclusion. The so-called "secondary gonocytes" as illustrated are smaller than certain other germ-cells designated "primary gonocytes," but no unequivocal transitions appear between the mesenchyme cells of the sexual gland or its peritoneal cells and the secondary gonocytes.

In a postscript (p. 33) Allen (1911) records preliminary results in the case of *Necturus* and *Amblystoma* similar to those reported by Dustin for *Triton*, namely, an actual mesodermal origin of sex-cells. Allen disposes of the conflicting views regarding the origin of germ-cells in amphibia by the claim that in the urodeles the usual source of origin of the germ-cells is the mesoderm; that in the anurans the source of origin is the entoderm; at the same time, he admits the possibility of exceptions.

A similar mesodermal origin of the sex-cell is described also for the teleost *Lophius* (Dodds, 1910). Wheeler's results on *Petromyzon* (1899) indicate that the germ-cells may become included in the mesoderm at the time it separates from the entoderm. Jarvis (1908) reports an entodermal origin of the primordial germ-cells also in the horned toad, *Phrynosoma cornutum*.

Gatenby (1916) has reexamined the evidence in the case of *Rana* (*temporaria*), *Salamandra*, *Triton* (*molge*), and *Amblystoma*. The observations on these forms accord in all the main points and "resemble *Bufo* in the history of germ-cell production," which it is claimed proceeds by a differentiation of germ-cells ("secondary gonocytes") from the germinal epithelium, in accord with Waldeyer's original theory. Gatenby thus disagrees with the conclusions of King (1908) for *Bufo lentiginosus*, Allen (1907) for *Rana pipiens*, and Kuschakewitsch for *Rana esculenta;* but agrees with the conclusion of Dustin (1907) for *Triton*, *Rana*, and *Bufo*, and with the earlier conclusion of Kuschakewitsch (1907) for *Rana pipiens*.

Gatenby, however, admits "that some germ-cells possibly migrate from the entoderm of the yolk-sac," but "feels sure that during life very large additional reinforcements of germ-cells arise in the epithelium of the gonad of Amphibia" (p. 276). His criticism, then, of the work of King, Allen, Beard, and others who hold to the view of a genetic independence between cœlomic epithelium and germ-cells is that they have studied only the earlier periods of the ontogeny. But in the male chick Swift has now (1916) examined also the young post-incubation stages, as late as 10 days, and still finds all the evidence in accord with the segregation view.

Gatenby describes the tadpole ovary as formed of (1) germ-cells of peritoneal origin; (2) germ-cells of retro-peritoneal origin; (3) germ-cells of endoderm (yolk-sac) origin. He inclines to believe that the germ-cells of the yolk-sac are all laid at the first spawning. He describes and illustrates intermediate stages between peritoneal cells and young germ-cells, but one is forced to the criticism that his figures give no adequate support to his claim of a transformation of mesoderm-cells into germ-cells.

In the lizard embryo (*Lacerta agilis*), according to von Berenberg-Gossler, the migration of the "primordial germ-cells" is not so strongly limited to the early developmental stages as described, *e. g.*, for *Chrysemys;* and the cells ("entodermal wandering cells") are relatively much more numerous; nor do any of them disintegrate; they become converted into mesoderm-cells. "The migration of cells out of the entoderm in *Lacerta* has no other significance than a dilatory development of mesoderm from entoderm" (pp. 247–248). He extends this interpretation to cover the so-called extra-regional genital cells of reptiles and mammals. As mesodermal cells they may possibly metamorphose into cells of the cœlomic epithelium, as earlier maintained by Hoffman. They wander from the entoderm largely to the location of the future pronephric (Wolffian) duct, to the construction of the caudal portion of which they greatly contribute. Occasional cells are found also in the somatopleure. None of the cells while within the mesoderm were found in process of mitosis. All these cells are believed to become changed into mesoderm-cells; thus they apparently disappear, but from some of those scattered among the cells of the peritoneal epithelium definitive germ-cells arise. This interpretation eases the difficulty of accepting the view of certain investigators (*e. g.*, Firket, Dustin, Gatenby, Felix) that both primary and secondary genital cells are formed, the primary becoming displaced through "degeneration" by the secondary. The whole genital gland would then arise from mesoderm, as maintained by some of the earlier investigators.

No attempt is made by von Berenberg-Gossler to apply the above interpretation to conditions in the chick, where these cells during early stages have an intravascular distribution. However, he expresses doubt regarding the germ-cell nature of these intravascular "germ-cells," many of which are in process of degeneration. A common interpretation of the phenomena relating to these "entodermal wandering cells" or "primordial germ-cells" in the lizard and the chick is obviously difficult if not actually impossible on this basis.

Rubaschkin (1908, 1909) describes an entodermal origin of primordial germ-cells also in rabbit and guinea-pig embryos; and he traces a migration route practically identical with that here described for *Caretta* and that described for *Chrysemys* by Allen. In the cat embryo, Winiwarter and Sainmont (1909) describe similar sex-cells in the embryonic ovary, but they interpret these cells as hypertrophied forms of the ordinary cells of the sex-cords.

Nagel (1889) described extra-regional germ-cells also in the human embryo, located near the pronephric duct. In 1911, Felix likewise described them in human embryos. In 1912, Fuss published his results of a study of 3 human embryos (of 2, 3, and 4 weeks) and 17 other mammalian embryos, including rabbit embryos and pig embryos (of 7 to 14 mm.). In the human embryo the sex-cells are said to be capable of amœboid motion; this movement is combined with a passive migration, the result of growth in the enveloping tissues. Fuss declares that aside from the germinal path there are no germ-cells, and that the so-called "germinal epithelium" plays no large rôle in the origin of germ-cells in mammals. The germ-cells are said to be present long before the differentiation of the sexual gland takes place. They arise from the entoderm, and after closure of the gut they migrate through the mesentery to the genital gland—in the human embryo about the fourth week, in the rabbit embryo about the thirteenth day, and in the pig embryo of about 14 mm. On entrance into the gland they may undergo active mitotic proliferation.

DISCUSSION AND CONCLUSIONS.

It would seem that the evidence is now sufficient to amply support the hypothesis of the extra-regional origin of the germ-cells first clearly enunciated by Nussbaum (1880) in opposition to the teaching of Waldeyer (1870), who derived the germ-cells by process of differentiation from the peritoneal epithelium. Swift's most recent publications (1915, 1916) clear also the additional obscurity regarding the germ-cells produced by Felix, who claimed that the primordial germ-cells ("primary genital cells") in amniotes, including man, have an extra-regional origin, but later disappear and become replaced by "secondary genital cells," the definitive germ-cells, differentiation products of the peritoneal epithelium. This is also the view of Dustin regarding certain amphibia and *Chrysemys*, and of Gatenby for the frog. Swift has shown for the chick that the sex-cords arise as ingrowths of proliferating regions of peritoneal epithelium into the subjacent mesenchyma of the genital fold (beginning about the sixth day of incubation), and that the sex-cells which have migrated from extra-regional areas to the peritoneal epithelium become involved in the sex-cords, where as oögonia and spermogonia they undergo proliferation and further differentiation.

Considering all the forms studied, from *Ascaris* to man, the evidence is all but complete for a morphologic continuity of the germ-cells, a "Keimbahn," or germinal path. According to Buchner (1910) no evidence appears for such a germinal path in annelids, mollusks, and echinoderms, nor according to Fick (1906) for plants. But these are simply instances regarding which our evidence is as yet very incomplete.

The evidence derived from this study of the embryo of the loggerhead turtle supports the theory of extra-regional origin of germ-cells and, so far as it goes, the Keimbahn theory. The facts are most closely in line with those reported by Allen for *Chrysemys*, by Woods for the dog-fish, and by Beard for the skate.

This work had in view three chief objects: (1) to discover the origin of the germ-cells and the route and manner of possible migration; (2) a careful examination of the cellular contents of all blood-channels with the expectation of removing a possible confusion between germ-cells and certain blood-cells; (3) to find a basis for harmonizing the results of von Berenberg-Gossler in the case of *Lacerta* with those reported for other reptiles, and possibly other vertebrate forms.

My first surprise was to find the facts in *Caretta* closely like those described by Allen for *Chrysemys*. The germ-cells are segregated in the entoderm from about the second to fifth day near the lateral border of the area pellucida, from a point near the cephalic tip of the pronephros to the caudal extremity. Up to the fourth day occasional cells occur also in the area opaca near the border of the area pellucida. This indicates the first step in the migration, namely, from area opaca to lateral border of area pellucida caudally; since germ-cells disappear later from the area opaca. In the area pellucida the germ-cells then become fairly closely aggregated into two bilateral cords. These are, however, not of uniform thickness throughout, but have an interrupted or segmental character, suggesting "gonotomes."

At about the fifth day germ-cells wander into the splanchnic mesoderm and migrate medially. When the hind-gut becomes closed (from the sixth to the tenth day) germ-cells become included in its mucosa and in the more peripheral portion of wall. From here many migrate through the mesentery across the angle of the cœlom to the differentiating sex-gland at the seventh day, a few apparently degenerating within the gut-wall. At the thirty-second day they are distributed among the peritoneal epithelium of the developing sex-gland and in the subjacent mesenchyma. Only very occasionally is a cell found extra-regionally at this stage, and apparently no sex-cords have as yet begun to form; but neither is there the slightest evidence of a differentiation of the so-called germinal epithelium into genital cells. In the earlier stages a few germ-cells may be seen in mitosis and an occasional cell may apparently divide at any stage.

My second surprise was to find practically no germ-cells in any vascular channels; only two exceptions were noted. Nor does there seem to be any possibility of confusion of sex-cells with blood-cells, judging from Swift's figures or on the basis of my sections of the loggerhead-turtle embryo. Swift's observations have now been confirmed by von Berenberg-Gossler for chick and duck. Von Berenberg-Gossler was unable, however, to find similar conditions in the case of the lizard embryo. The condition of abundant intravascular germ-cells in the chick embryo and the duck embryo is so far unique. Conditions respecting the germ-cell history in birds are evidently different from those obtaining in reptiles and certain other amniotes, and depend upon the cephalic (pro-amniotic) site of origin of the germ-cells and their close relation to the vascularizing mesoderm.

Considering the initial very close relationship (both spatial and genetic) between the entoderm and the mesoderm at the time when the latter is in the early stages of vascularization and the considerable migratory capacity of the primordial germ-cells, the presence of these cells outside of the usual migration route, even in the blood-vessels, is nothing extraordinary. In the chick the initial source of origin is in the entoderm at the anterior extremity of the blastoderm in a region originally free of mesoderm. The cells begin their first migration about the time the vascularizing mesoderm invades this region, and so readily enter into the blood-vessels by which they are transported in large part caudally to the medial splanchnopleure, whence they pass via the mesentery to the gonads (Swift). In forms like the turtle and the dog-fish, where the germ-cells are originally scattered caudally in the area opaca, they do not in large numbers come into so intimate early relation with the young blood-vessels, and in consequence reach the medial splanchnopleure largely via the visceral layer of the mesoderm by their own amœboid activity, meanwhile passing through a temporary stage of sharp segregation into the paired cords of the area pellucida. But even here it is apparently an easy matter for the germ-cells to become involved with the blood-channels either through active migration or passive inclusion. There is nothing, therefore, inherently contradictory or unique in the condition of the germ-cell migration as it obtains in the chick and duck. The difference is one of degree rather than kind, and depends upon the difference in initial location of the primordial germ-cells with respect to the mesoderm and the head end of the embryo.

The very careful work of Allen, in which he counted the number of germ-cells at the different stages of development in different areas in *Chrysemys*, clearly shows that the total number of germ-cells in different individuals of a species varies within wide limits (302 to 1,744), and that the time and rate of migration also vary considerably. I therefore made no attempt to approach the question of the route and manner of migration on the basis of germ-cell counts. The fact that at progressively later stages in development the germ-cells were found in the entoderm near the lateral border of the area pellucida, then in the visceral plate of the lateral mesoderm and more medially in the entoderm (later also in the closed gut and enveloping mesenchyma), then in the mesentery, and finally across the cœlomic angle among the peritoneal epithelium of the sexual gland (genital fold), indicates that this represented the actual route of migration. The further facts that only an occasional germ-cell was seen in mitosis throughout the series (second to thirty-second day), that only a few were seen in conspicuous stages of degeneration, and that practically none appeared in blood-vessels (and only a few in the periaortic mesenchyma) seems to prove the indicated route as the actual and substantially exclusive migration path. In the case of *Cymatogaster*, also, Eigenmann found that the sex-cells do not divide from the period before the first somites have formed—at which time they are first distinguishable—up to about the period when the young fish reaches a length of 7 mm.

The close similarity, which in early stages amounts practically to an identity, between the primordial germ-cells and the cells of the entoderm (especially the yolk-sac entoderm) need not, and almost certainly does not, imply a derivation of germ-cells from entoderm-cells. In the first place, the germ-cells can always be distinguished from the entodermal cells (in spite of structural and tinctorial similarity between the cells) by the rounded (spherical or oval) form of the germ-cells. The cells of the yolk-sac entoderm, which they resemble most closely, are of irregular shape. Occasionally a germ-cell may become wedged in between the entoderm-cells and so forced into very intimate cytoplasmic relationship and into irregular form, in which condition it may be impossible to distinguish between the two; but the resemblance signifies simply, most probably, a similar low grade of differentiation from the original blastomeres. The germ-cells are therefore not *derived* from the entoderm-cells by differentiation. These two types of cells are similar because they are practically similarly undifferentiated, both containing a large, finely granular, pale nucleus and a large cytoplasmic content of yolk-globules. The germ-cells simply remain *dispersed* among the entodermal-cells after the segmentation stages and from here migrate via the area pellucida to the mesentery and thence to the gonads. The germ-cells here, as in certain invertebrates (*e. g.*, *Ascaris*, Boveri) most probably have been held apart from the soma-cells since an early segmentation stage and then, from widely scattered areas, have migrated through progressively more sharply segregated foci to the gonads, maintaining thus a continuous germ-cell cycle from blastomeres (perhaps a particular portion of the fertilized egg, as, for example, the pole disk of certain Diptera, Hegner) to the gonocytes of the definitive genital glands.

It remains to discuss the mode of the extravascular migration—*i. e.*, whether active (amœboid) or passive, or both. A passive migration is strongly urged by von Berenberg-Gossler. He thinks the germ-cells are passed from the lateral to the medial entoderm, and from the lateral visceral mesoderm to the mesentery, and then across the cœlomic angle by the mechanical process of unequal growth. Growth is no doubt a factor in the shifting. But the chief factors inhere in the cells themselves, which (judged from their shape and the manner in which the mesenchymal cells are compressed in the direction of a path towards the root of the mesentery) is an active amœboid movement of the cells. The cells evidently move medially both within the endoderm and in the mesoderm. Such as lie medially in the entoderm become inclosed within the hind-gut, whence the majority later migrate into the mesentery.

Only two germ-cells were found at any stage within any blood-channels. But no confusion between blood-cells (hemoblasts) and germ-cells seems possible. The germ-cells are at all stages larger than the largest granulocyte (eosinophil, whose granules also stain black in iron-hematoxylin) and the largest hemoblast, the character and staining capacity of whose nucleus is very different. There is therefore no room for doubt that in chick (Swift) and duck (von Berenberg-Gossler) the germ-cells actually do migrate (passively) towards the genital ridge largely through the blood-vessels. The locus of original segregation in birds is such as to provide in the blood-channels the most favorable method for the longer portion of the route of migration to the sexual gland.

As Allen has already suggested, the reason for the mesodermal origin of the germ-cells in urodeles and possibly certain other vertebrates may be a relatively early separation of mesoderm from entoderm—that is, the germ-cells of the primitive entoderm may have become separated with the portion which contributed to the splanchnic layer of the mesoderm before they could migrate from the entoderm proper.

In *Lacerta* von Berenberg-Gossler describes cells comparable to the so-called primordial germ-cells passing from the entoderm to both layers of the mesoderm, and to the dorsal layer of the intermediate cell-mass, where caudally the cells are said to contribute largely to the formation of the Wolffian duct. Von Berenberg-Gossler interprets his results to mean a belated origin of mesoderm from entoderm. He very ingeniously suggests that these "germ-cells" wander also to the sex-gland, where they may transform into mesenchymal-cells, and then retransform into germ-cells, but presents no histologic data in support. Von Berenberg-Gossler's results and his interpretation are unique and do not seem to be capable of being brought into harmony with the results obtained in any other form thus far studied, nor with any common interpretation. Von Berenberg-Gossler's interpretation is more or less plausible, but can not be said to be wholly satisfactory. The condition in the lizard may be simply an exaggeration of that in other forms where a certain number of germ-cells go astray during the period of migration.

Since germ-cells can migrate by their own activity, it is not difficult to conceive of their presence anywhere, even in the ectoderm, and certainly not in the developing Wolffian duct, where, unless they retrace their course towards the genital gland, they may undergo degeneration (or possibly lie dormant until a favorable influence may excite to the production of a tumor or a teratoma), but that they should contribute to the formation of the Wolffian duct is thus far an anomalous phenomenon.

When one considers in common conditions in the chick, lizard, toads and frogs, salamanders, and turtles, it becomes clear that there is great variation in the manner in which the comparable elements (the "primordial germ-cells") arise, migrate to the sexual gland, and develop. Underlying these discrepant phenomena, however, there appears a fundamental harmony, namely, an early segregation of germ-cells from somatic cells, a germinal path from blastomeres to genital cells of the genital gland, probably from blastomeres to sperm and ova; a migration from the entoderm (or its derivative, the mesoderm, in urodeles and lamprey), through the splanchnic layer of the mesoderm to the mesentery and thence to the gonads (with possible aberrant migrants anywhere).

And in the light of Swift's latest researches in the case of the sex-cords and the ova of the chick ovary and the spermatozoa of the testis, it now seems clear (notwithstanding the contradictory findings of Dustin in *Chrysemys* and of Gatenby in the frog) that the definitive germ-cells have had an extra-regional origin and do not arise by differentiation from germinal epithelium (cœlomic epithelium), but become involved in columnar invaginations of these cells to form sex-cords. Certain observations (*e. g.*, those of Felix, Dustin, and Firket) to the effect that the primordial germ-cells ("primary genital cells") degenerate early and are replaced by cœlomic derivatives in the genital gland, may possibly be explained by the facts that in certain amniotic forms the primordial germ-cells degenerate relatively more extensively and that mitosis does not generally appear until the remainder reach the sex-glands, where it becomes active in those cells involved in the sex-cords formed by the "germinal epithelium." The great proliferative activity of the remaining primordial germ-cells in the formation of oögonia and the spermogonia, among the cells of the cœlomic epithelium, gives the appearance of an active differentiation and derivation from the cœlomic epithelial-cells. The products of this intense proliferation are relatively smaller than the earlier germ-cell progenitors, which phenomenon makes the superficial resemblance between growing cœlomic epithelial cells and the definitive germ-cells more close. At any rate, no clear case has yet been made out for the differentiation of germ-cells from cœlomic epithelium at any stage in any form.

There is no evidence in *Caretta* of a differential mitochondrial content between the germ-cells and somatic cells, as maintained by Tschaschin in the case of the chick embryo and by Rubaschkin in the case of certain mammalian embryos; and as denied by Swift and von Berenberg-Gossler. The germ-cells are distinguished from the somatic cells by their larger size, their generous yolk-content, their large vesicular oxyphilic nucleus, with its delicate, finely granular, radially arranged, nuclear network. These nuclei resemble more closely those of the entoderm-cells of the area opaca, indicating a low grade of differentiation of these two cells and a close genetic relationship inhering most probably in a common near ancestor, from which they have departed but little in their slight progressive differentiation. This indicates also that the germ-cell commonly remains undifferentiated until a relatively late period in its history, when it takes residence in the genital gland. Its load of nutritive material in the form of yolk (still present in the 25-day stage) also indicates a low grade of differentiation and suggests the cause of its lack of proliferative capacity until digestion of this yolk is completed, and the further reason of its great capacity for later proliferation and differentiation, especially in the male.

But many more researches covering the later periods of the history of the ovary with special reference to the so-called "germinal epithelium," such as those of Swift on the chick and of Gatenby on the frog, are needed before the hypothesis of a germinal path for vertebrates can be said to be completely established. In view of conditions in the gonads of certain annelids, mollusks, and echinoderms, where enormous numbers of germ-cells are formed during successive years, and the disagreements which still exist with respect to such relatively simple forms as frog (Allen and King *vs.* Dustin and Gatenby), chick (Swift *vs.* Firket), and the turtle, *Chrysemys* (Allen *vs.* Dustin), the claims that two series of genital cells occur—a primordial and a secondary or definitive—the one derived directly from a blastomere of the later cleavage stages and so segregated from the somatic cells, and the other derived by differentiation from the cœlomic epithelium of the genital ridge, and so modified soma-cells, while on logical grounds inherently improbable, and without firm observational basis—and disproved on histologic grounds, I believe, in the case of *Caretta*—can not perhaps be said to have been definitely disposed of.

SUMMARY OF RESULTS.

1. Twenty-five embryos of the loggerhead turtle (*Caretta caretta*), ranging from the second day (5 somites, 2 mm. length) to the thirty-second day of incubation, were employed in this investigation. The early history of the primordial germ-cells is very similar to that described by Allen for the turtle *Chrysemys* and by Woods for the dogfish.

2. The primordial germ-cells migrate during the second day from the yolk-sac entoderm, where they were more or less widely scattered caudally, into the lateral border of the area pellucida on each side of the embryonic disk. Here they become sharply segregated by the beginning of the third day into two bilateral cords situated in the entoderm of the area pellucida laterally in the caudal half of the disk. In the 2-day embryo they extend from the neurenteric canal to the end of the primitive streak; in the 3-day embryo from the sixth somite to the caudal extremity of the streak. The cords become more medially placed, make a linear connection with the overlying visceral mesoderm, and their cells migrate during the fifth day into this mesoderm, and thence medially (during the sixth and seventh days) towards the root of the forming mesentery of the closing hind-gut. Individual cells migrate medially also within, or back into, the entoderm of the gut. The germ-cells in the medial entoderm become included in the mucosa of the closed hind-gut, those in the mesoderm in the enveloping mesenchyma and the gut end of the mesentery. From these locations the majority of the germ-cells subsequently (seventh to twelfth day) migrate up the mesentery and across the cœlomic angle into the future sexual gland. They become incorporated among the mesenchymal cells of the gland and the covering peritoneal epithelium, where they suffer no striking change in form, size, or content at least as late as the thirty-second day of incubation.

3. The germ-cells migrate by amœboid activity, assisted in small part probably by the factor of unequal growth, involving the shifting of the medial portion of the splanchnopleure to the mesentery and the dorsal portion of the mesentery to the gonads.

4. The migration period is not sharply limited. It is at its height from the seventh to the twelfth day, and practically ceases about the sixteenth day. But occasional extra-regional cells may still be found in the gut and mesentery at the 32-day stage—usually, however, showing signs of degeneration.

5. A certain number of germ-cells migrate out of the regular germ-cell route and go astray. Such strays are especially numerous in the periaortic mesenchyma caudally, where they may become incorporated among the neuroblasts of the developing peripheral sympathetic ganglia. The majority of these strays probably degenerate *in situ*, but some may possibly persist to form, under the proper pathologic

stimulus, a focus of neoplastic growth. An occasional cell is found also in the blood-vessels of this region. Such may be carried by the blood-stream to distant regions and perhaps again enter the mesenchyma or degenerate within the vessels.

6. The total number of primordial germ-cells counted in a 12-day embryo is 352, the number within the gonads being about equally divided between the two (118 left to 127 right).

7. Occasional cells may divide by mitosis, or undergo degeneration, at any stage of their history or at any point of the route. Mitoses are more numerous during earlier stages and among the entodermal cells; degeneration is more general during the later stages and in the mesenchyma of the closed hind-gut.

8. No germ-cells were found contributing to the formation of the Wolffian duct. There is no evidence in this form in support of von Berenberg-Gossler's claim, on the basis of his observations on the lizard embryo, that the so-called primordial germ-cells represent simply a belated stage of mesoderm formation from entoderm.

9. The germ-cells do not differ from young somatic cells in the character of their mitochondrial content. The mitochondria include granular as well as beaded-rod and filamentous forms.

10. No transition stages between cœlomic epithelial cells and germ-cells appear up to the 32-day stage. From the 16-day stage on, when the nuclei of some of the germ-cells within the gonads become coarsely granular and the reticulum stains more deeply, apparent transition stages occur between the larger of the mesenchymal cells and the smaller included subepithelial germ-cells. But no secure histologic basis can here be found for separating the germ-cells of the gonads into large "primary genital cells" and smaller "secondary genital cells" (Felix) or "gonocytes" (Dustin) derived by process of differentiation from the cells of the "germinal" (peritoneal) epithelium or the subjacent mesenchyma. The size variations among the germ-cells of the gonads of the older stages are no greater than in the original cords of the area pellucida or in the subsequent early stages; and the cytologic similarity between the two dimensional grades of cells is much closer than between the larger mesenchymal cells and the smaller germ-cells.

11. The evidence derived from a study of the *Caretta* embryos is in complete harmony with the idea of a single uninterrupted line of sex-cells from primordial germ-cells to oögonia and spermogonia, and with the hypothesis of a vertebrate Keimbahn or continuous germinal path.

12. The variations in the distribution of the primordial germ-cells during earlier embryonic stages described by various investigators for a number of vertebrate forms—as pertains both to their presence in blood-vessels (chick, Swift; duck, von Berenberg-Gossler) and in various regions and tissue remote from the more direct and more usual germinal route (Wolffian duct and somatopleure in the lizard,

von Berenberg-Gossler; and sympathetic ganglia in the loggerhead turtle, Jordan); and to their apparent primary (urodeles) or secondary (anurans and other vertebrates) derivation from the splanchnic layer of the lateral mesoderm—are incidental to their original location with respect to the embryonic area and the vascularizing mesoblast of the blastoderm, and to their amœboid capacity. Since the primordial germ-cells are genetically directly related to neither of the secondary germ-layers, their origin in either (entoderm or mesoderm) has no fundamental significance. Since they are capable of amœboid activity, and may become included in blood-vessels, they may migrate anywhere, and so occur in any location, from where they may subsequently migrate again to the more direct germinal path, or perhaps disintegrate. The fact of fundamental significance with respect to the primordial germ-cells is their original extra-regional distribution and their direct genetic independence of the soma-cells.

BIBLIOGRAPHY.

Allen, Bennet M. 1904. The embryonic development of the ovary and testis of the Mammalia. Amer. Jour. Anat., vol. III, p. 189.

———. 1906. The origin of the sex-cells of *Chrysemys*. Anat. Anz., Bd. 29.

———. 1907*a*. A statistical study of the sex-cells of *Chrysemys marginata*. Anat. Anz., Bd. 30, p. 391.

———. 1907*b*. An important period in the history of the sex-cells of *Rana pipiens*. Anat. Anz., Bd. 31, p. 339.

———. 1911. The origin of the sex-cells of *Amia* and *Lepidosteus*. Jour. Morph., vol. 22, p. 1.

Beard, J. 1900. The morphological continuity of the germ-cells in *Raja batis*. Anat. Anz., Bd. 18.

———. 1904. The germ-cells. Part I. Jour. Anat. and Physiol., vol. 38.

v. Berenberg-Gossler, H. 1912. Die Urgeschlechtszellen des Hühnerembryos am 3. und 4. Bebrütungstage, mit besonderer Berüchsichtung der Kern- und Plasmastrukturen. Archiv f. mikr. Anat. Abteilung II, Bd. 81, s. 24.

———. 1914. Ueber Herkunft und Wesen der sogenannten primären Urgeschlechtszellen der Amnioten. Anat. Anz., Bd. 47, s. 241.

Boveri, Th. 1904. Ergebnisse über die Konstitution des chromatischen Substanz des Zellkerns. Fischer, Jena.

Buchner, P. 1910. Die Schicksale des Keimplasmas der Sagitten in Reifung, Befruchtung, Keimbahn, Ovogenese, und Spermatogenese. Festschr. f. R. Hertwig.

Dantschakoff, V. 1908. Entwicklung des Blutes bei den Vögeln. Anat. Hefte, Bd. 37.

d'Hollander, F. 1904. Recherches sur l'oogenese et le noyau vitellin de Balbiani chez les oiseaux. Arch d'Anat. micr., T. 7.

Dustin, A. P. 1907. Recherches sur l'origine des gonocytes chez les Amphibiens. Arch. de Biol., T. 23, p. 411.

———. 1910. L'origine et l'evolution des gonocytes chez les Reptiles. Arch. de Biol., T. 25, p. 495.

Eigenmann, C. H. 1892. On the precocious segregation of the sex-cells in *Cymatogaster aggregatus* Gibbons. Jour. Morph., vol. 5, p. 481.

———. 1897. Sex-differentiation in the viviparous teleost *Cymatogaster*. Arch. f. Entw.-Mech., Bd. 4, s. 125.

Felix, W. 1906. Die Entwicklung der Keimdrüsen und ihrer Ausführungsgänge. Handb. d. Entw. von O. Hertwig.

———. 1911. Keibel and Mall's Human Embryology, vol. II, chap. XIX, p. 882.

Fick, R. 1906. Vererbungsfrage, Reduktions- und Chromosomenhypothesen, Bastardregeln. Ergebn. d. Anat. u. Entw., Bd. 16.

FIRKET, JEAN. 1914. Recherches sur l'organogenèse des glandes sexuelles chez les oiseaux. Arch. de Biol., T. 2.

FUSS, A. 1911. Ueber extraregionäre Geschlechtszellen bei einem menschlichen Embryo von vier Wochen. Anat. Anz., Bd. 39.

———. 1912. Ueber die Geschlechtszellen des Menschen und der Säugetiere. Archiv f. mikr. Anat. Abteilung II, Bd. 81, s. 1.

HAECKER, V. 1897. Die Keimbahn von Cyclops. Neue Beiträge zur Kenntnis der Geschlechtszellensonderung. Arch. f. mikr. Anat., Bd. 49.

HASPER, M. 1911. Zur Entwicklung der Geschlechtsorgane von Chironomus. Zoolog. Jahrb. f. Anat. u. Ont., Bd. 31.

HOFFMAN, C. K. 1892. Étude sur le dévéloppement de l'appareil urogénitale des oiseaux. Verh. kon. Ak. d. Wetensch., Amsterdam.

JARVIS, M. 1908. The segregation of the germ-cells of *Phrynosoma cornutum*. Biol. Bull., vol. 15.

JORDAN, H. E. 1908. The relation of the nucleolus to the chromosomes in the primary oöcyte of *Asterias forbesii*. Papers from the Marine Biological Station at Tortugas; Carnegie Inst. Wash. Pub. No. 102.

———. 1914. Sex chromosomes in *Chelonia*. Science, vol. 39 p. 1003.

———, and J. C. FLIPPIN. 1913. Hæmatopoiesis in Chelonia. Folia Hæmatologica, Bd. 15, s. 1.

KING, H. D. 1908. The oogenesis of *Bufo lentiginosus*. Jour. Morph., vol. 19, p. 369.

KUSCHAKEWITSCH, S. 1908. Ueber den Ursprung des Urgeschlechtszellen bei *Rana pipiens*. Stzber. math. phys. Klasse, K. bayer Akad. Wiss., Bd. 38.

———. 1910. Die Entwicklungsgeschichte der Keimdrüsen von *Rana esculenta*, ein Beitrag zum Sexualitätsproblem. Festschr. f. R. Hertwig, Jena.

MINOT, CH. S. 1894. Gegen das Gonotom. Anat. Anz., Bd. 9.

NAGEL, W. 1889. Ueber das Vorkommens von Primordialeiern ausserhalb der Keimdrüsenanlage beim Menschen. Anat. Anz., Bd. 5.

NUSSBAUM, M. 1880. Zur Differenzierung des Geschlechts im Tierreich. Arch. f. mikr. Anat., Bd. 18.

———. 1901. Zur Entwicklung des Geschlechts beim Huhn. Verh. Anat. Ges. zu Bonn.

RUBASCHKIN, W. 1907*a*. Ueber das erste Auftreten und Migration der Keimzellen bei Vögelembryonen. Anat. Hefte, Bd. 35, p. 243.

———. 1907*b*. Zur Frage von der Entstehung der Keimzellen bei Säugetierembryonen. Anat. Anz., Bd. 31.

———. 1909. Ueber die Urgeschlechtszellen bei Säugetieren. Anat. Hefte, Bd. 39.

———. 1910. Chondriosomen und Differenzierungsprozesse bei Säugetierembryonen. Anat. Hefte, Bd. 41.

SEMPER, C. 1875. Das Urogenitalsystem der Plagiostomen und seine Bedeutung für dasjenige der übrigen Wirbeltierre. Arb. zool.-zoot. Inst., Würzburg.

SWIFT, C. H. 1914. Origin and early history of the primordial germ-cells in the chick. Amer. Jour. Anat., vol. XV, p. 483.

———. 1915. Origin of the definitive sex-cells in the female chick and their relation to the primordial germ-cells. Amer. Jour. Anat., vol. XVIII, p. 441.

———. 1916. Origin of the sex-cords and definitive spermatogonia in the male chick. Amer. Jour. Anat., vol. 20, p. 375.

TSCHASCHIN, S. 1910. Ueber die Chondriosomen der Urgeschlechtszellen bei Vögelembryonen. Anat. Anz., Bd. 37.

WALDEYER, W. 1870. Eierstock und Ei. Leipzig.

———. 1906. Die Geschlechtszellen. Im Handb. d. Entw. von O. Hertwig.

WEISMANN, A. 1902. Vorträge über Descendenztheorie. Jena.

WHEELER, W. M. 1900. The development of the urogenital organs of the lamprey. Zool. Jahrb., Anat. Abth., Bd. 13.

v. WINIWARTER, H., and G. SAINMONT. 1908. Nouvelle recherches sur l'ovogenèse et l'organogenèse de l'ovaire des Mammifères (chat.). Arch. Biol., T. 24.

WOODS, F. A. 1902. Origin and migration of the germ-cells in Acanthias. Amer. Jour. Anat., vol. 1, p. 307.

DESCRIPTION OF ILLUSTRATIONS.

FIG. 1.—Composite drawing of left side of mesentery near its root in the 11-day embryo. All the cells shown are from the immediate vicinity of the same section. *M*, mesothelium of mesentery; *h* and *e*, hemoblast and erythrocyte, respectively, in the aorta; *b*, eosinophil blood granulocyte, lying among the mesenchymal cells; *a*, typical primordial germ-cells (primary genital cells), laden with yolk-globules; *i*, attraction sphere, shown both in the granulocyte and the genital cell. Similar germ-cells appear also in this and immediate sections in the gut (both among the entodermal cells and in the enveloping mesenchyma) and among the mesothelial cells of both the mesentery and the genital ridge. This section is very similar to that of figure 2 (plate V). Flemming fixation, iron-hematoxylin stain; magnified 1,000 diameters.

FIG. 2.—Portion of genital ridge of same 11-day embryo, showing a germ-cell among the flattened cells of the peritoneal epithelium; the germ-cell contains many mitochondria, of granular, rod, beaded-rod, and a few of filamentous forms; also some yolk-granules. *b*, an eosinophil granulocyte. ×1,000.

FIG. 3.—Section through cœlomic angle of same 11-day embryo, showing a germ-cell among the peritoneal epithelial-cells of both the mesentery (*aa*) and the genital ridge (*ac*); the germ-cell *ab* lies among the mesenchymal cells of the mesentery, apparently in process of migration into the mesothelial layer. Both *aa* and *ac* also are in amœboid activity. Cell *ac* is loaded with an apparently continuous mass of yolk material. Magnified 1,000 diameters.

FIG. 4.—Germ-cells from the 10-day embryo (similar to fig. 2, plate V), in the region of the hind-gut. (Helly fixation, Giemsa stain.) *a*, germ-cell from the entoderm of the gut (the nucleolus stains a deep violet, the nucleus, with its granular reticulum stains a light bluish-pink, and the reticular vacuolated cytoplasm stains a pink color); *b*, germ-cell in mesenchymal layer of gut; *c*, germ-cell in the peritoneal epithelium of the genital ridge; *d* and *e*, germ-cells from the mesenchyma of the developing genital gland; *f*, eosinophil from mesentery (granules deep red, nucleolus blue). ×1,000.

FIG. 5.—Erythroblast within a blood-vessel, in the 11-day embryo. ×1,000.

FIG. 6.—Germ-cells from 12-day embryo. (Helly fixation; iron-hematoxylin stain.) *a*, germ-cell from among the entodermal cells of the still open portion of the hind-gut (fig. 1, plate VI); *i*, attraction sphere; *b*, germ-cell from among the peritoneal epithelium of the genital ridge (the nucleolus is stained a deep black, the granular nucleus a brownish gray, the vacuolated cytoplasm a light gray); *c*, germ-cell from middle of mesentery closely enveloped by flattened mesenchymal cells, simulating a close-fitting capsule. ×1,000.

FIG. 7.—Germ-cell from "germinal epithelium" of genital-gland of 22-day embryo. ×1,500. Helly fixation; stained *in toto* with Delafield's hematoxylin. The nucleolus stains a deep blue, the granular nucleus light blue, and the reticluar cytoplasm a still lighter blue.

FIG. 8.—Germ-cell from subepithelial mesenchyma of genital gland of 25-day embryo. The cell is closely enveloped by flattened mesenchymal cells, simulating a capsule. ×1,500.

FIG. 9.—Germ-cell from submesothelial mesenchyma of sexual gland of 32-day embryo. ×1,500.

FIG. 10.—Germ-cells and blood-cells from the 8-day embryo. *a*, germ-cell from the splanchnic layer of lateral plate of mesoderm, near the border of the area pellucida; *b*, germ-cell from mesentery closely enveloped by flattened mesenchymal cells; *c* and *d*, hemoblasts from mesochyma of mesentery and from the aorta respectively. ×1,500.

FIG. 11.—Section of entoderm of open gut of 7-day embryo from near the border of the area pellucida, showing a germ-cell among the entodermal cells. The cytoplasm of the germ-cell contains large irregular peripheral vacuoles, due to the solution of the yolk-content. A cell from the superjacent mesenchyma is shown at (*a*) for purpose of comparison between the nuclei of the germ cells, entodermal cells, and more differentiated mesenchymal cells. ×1,500.

FIG. 12.—Germ-cell from entoderm of dorsal region of hind-gut, of a 7-day embryo. The cytoplasm contains enormous peripheral vacuoles, where the yolk-globules have been dissolved. ×1,500.

FIG. 13.—Germ-cell in the entoderm from about mid-line of area pellucida in a 3-day embryo. The cell is in the act of migrating into the superjacent visceral layer of the lateral plate of mesoderm. ×1,500. The arrow points towards the embryonic axis.

FIG. 14.—Germ-cell of a 3-day embryo (fig. 1, plate III), from area pellucida near its lateral border. The cytoplasm contains two large vacuoles where some of the yolk material has been dissolved. This cell has a diameter of 16.5 microns. The germ-cells vary from 13 to 22 microns in diameter.

FIG. 15.—Germ-cell from area opaca, near the margin of the area pellucida, of a 3-day embryo. For comparison a nucleus from the entodermal syncytium is added at *a*. Note the close similarity of structure. The cytoplasm of the germ-cell contains large spherical yolk-globules, which at this stage of development were not dissolved in the alcohols after Helly fixation (iron-hematoxylin stain). Only at later stages, when the yolk has suffered further changes in the process of elaboration for nutritive purposes, does it become susceptible to the solvent action of the alcohols. ×1,500.

XIV.

ATRESIA OF THE ESOPHAGUS IN THE EMBRYO OF THE LOGGERHEAD TURTLE (CARETTA CARETTA): A NORMAL DEVELOPMENTAL CONDITION.

BY H. E. JORDAN,
Department of Anatomy, University of Virginia.

Four plates of nine figures.

ATRESIA OF THE ESOPHAGUS IN THE EMBRYO OF THE LOGGERHEAD TURTLE (CARETTA CARETTA): A NORMAL DEVELOPMENTAL CONDITION.

By H. E. Jordan.

INTRODUCTION.

In a series of embryos of the loggerhead turtle collected and used originally for a study of the history of the primordial germ-cells, it was noticed that the esophagus was solid for a greater or less extent, approximately from the point of origin of the respiratory anlage to its bifurcation into the bronchi, from the eleventh to the thirty-second day of incubation. At the latter stage the esophagus is still occluded at its oral end, though now fenestrated for a considerable extent caudally; and it seems probable that the esophageal atresia persists practically to near the end of the incubation period (8 weeks) at the level just behind the opening of the larynx.

The points of special significance in regard to this material are: (1) the relatively longer persistence of the occlusion than has yet been described for any other form; (2) the absence of contributory yolk in the stenosed area; (3) close relation to the point of origin of the respiratory anlage, which fact may disclose its possible functional significance.

Balfour was the first (1878) to describe a similar phenomenon in the esophagus of certain selachii. Kreuter (1903) confirmed these observations in the case of *Pristiurus* and *Torpedo*. Dean (1897) reports a solid esophagus in larvæ of *Amia calva*. An occluded esophagus is said to occur also in certain bony fishes—*e. g.*, herring, trout, salmon (Balfour, Oppel). In cyclostomes the esophagus remains patent throughout development (Kreuter). In certain amphibia (*Bufo, Rana*) the esophagus becomes occluded, in part through the medium of contributory yolk-globules (Meuron, 1886); and the same is true for certain reptiles (*Anguis fragilis*, Oppel, 1891; *Lacerta*, Meuron, 1886). According to Meuron, the esophagus of the chick embryo of the fifth day is occluded for a length of 115 microns, but regains partial patency again in the sixth day through the appearance of vacuoles. Lillie describes the esophagus of the chick embryo as completely occluded immediately behind the glottis from the eighth to the eleventh day, "owing to proliferation of the lining cells."

Kreuter (1905) was the first to describe an epithelial obliteration of the esophageal lumen in the human embryo, contrary to the teaching of Kollmann and other embryologists that no solid stage of the esophagus occurred in mammals and in man. Kreuter describes also similar obliterated areas in the mid-gut and hind-gut of fetuses between the fourth and tenth weeks. In four human embryos, measuring from 8.4 to 16 mm., Lewis (1912) describes an esophagus whose lumen is pervious throughout; however, he describes vacuoles in the epithelial lining of these stages similar to those described by Kreuter as stages in the opening of the solid esophagus. But he regards an atresia of the esophagus in the human embryo as abnormal at all stages (p. 368).

It would seem that an embryonic normal atresia of the esophagus is a widespread phenomenon among vertebrates, and is essentially similar from elasmobranch fishes to man.

The phenomenon has not yet, as far as I am aware, been described for turtles, a circumstance which adds to the interest of this investigation; nor have its intimate spatial relationship to the respiratory anlage and its probable functional significance been hitherto pointed out.

This work is based chiefly on embryos of the loggerhead turtle, *Caretta* (*Thalassochelys*) *caretta*, but enough has been seen also in embryos of the snapping turtle (*Chelydra serpentina*) and of *Chrysemys marginata* to warrant the statement that in these forms a substantially identical process occurs.

In occasional pig embryos also, from 8 to 12 mm. length, I have noticed short areas of occlusion in the esophagus.

More or less extensive atresia occurs also in the stomach, duodenum, and other portions of the small intestine, large intestine, and rectum in certain of the above-named forms, especially in mammals. Of these regions the duodenum is most commonly occluded. The character of the occlusion and the manner of the reestablishment of patency are in general here also through the agency of originally discrete interepithelial vacuoles which gradually coalesce. In the turtle *Caretta* no other portions of the alimentary tract were at any stage similarly occluded.

MATERIAL.

The material consists of a series of 26 loggerhead-turtle embryos ranging from the second to the thirty-second day of incubation. It was collected in the summer of 1914 during a visit to the Laboratory of Marine Biology of the Carnegie Institution of Washington, located on Loggerhead Key, Florida. I am greatly indebted to Dr. Alfred G. Mayer for the excellent facilities afforded for securing and preserving a large number of specimens, approximately 200.

Two fixing fluids were employed almost exclusively, namely, the Zenker-formol modification of Helly and the strong chrom-aceto-osmic solution of Flemming. After the Helly fixation, different sets of sections were variously stained with the Giemsa solution, the iron-hematoxylin mixtures of Heidenhain, and the Delafield's hematoxylin and eosin combination.

DESCRIPTIVE.

In embryos up to the end of the eleventh day the lumen of the esophagus is still patent, but the epithelial lining is greatly thickened dorsally and the lumen is in consequence constricted (fig. 1).[1] Dorsally the lining epithelium consists of about 7 layers of spheroidal cells; ventrally it is only 3 to 4 layers thick, and the cells are cylindrical. Figure 1 is taken at the level of the oral end of the laryngo-tracheal evagination. Figure 2 is taken 150 microns caudally, at the point of bifurcation of the trachea into the bronchi. These levels are important landmarks, for they are approximately the limits of the later atresia. It should be noted also that the laryngo-tracheal anlage at this stage is patent throughout; at later stages the epithelial thickening may occasionally involve also its orifice and cause a temporary occlusion. Mitosis is very extensive among the entodermal lining-cells of this region, especially centrally.

By the end of the twelfth day the esophagus is practically solid (fig. 3) from the level of the origin of the tracheal anlage to the point of its bifurcation, about 250 microns. A minute central opening, approximately the diameter of a nucleus of the surrounding cells, may remain. By the thirteenth day this opening also has become closed. At this stage the trachea is open throughout. This stage is of prime importance, for it shows the manner of closure of the esophageal tube.

During succeeding days the extreme proximal end of the trachea (larynx) becomes solid, as well as the esophagus between the limits of the origin and bifurcation of the trachea. At the end of the sixteenth day the esophageal atresia extends through 1,680 microns, from a medial pharyngeal stenosis 300 microns behind the point of origin of the laryngo-tracheal tube to 720 microns behind the point of its bifurcation. Figure 4 is of the cephalic extremity of the atresia, and shows also the atretic condition of the trachea proximally. Figure 5 is of a section 300 microns caudal to figure 4. Vacuoles occur among the epithelial cells centrally; such are present to the point of transition into the stomach through 1,380 microns (fig. 6).

In the 25-day embryo the esophagus is closed through 2,650 microns. The initial point of closure is medially just behind the laryngeal opening. As compared with earlier stages (*e. g.*, the 16-day embryo), it seems that the cephalic extremity of earliest closure—*i. e.*, *over* or just behind the tracheal opening—has shifted slightly caudally so as to leave the trachea (larynx) freely pervious anteriorly to the closed esophagus. The medial (pharyngeal) closure rapidly spreads laterally, so that within 100 microns the laterally wide, dorso-ventrally much compressed esophagus is completely closed, except for the lateral extremities, which contain a small central aperture. These lateral apertures persist through about 600 microns, the extremities having meanwhile bent ventrally, giving the esophagus through about 600 microns a wide crescent-shape, about 200 microns behind which point vacuoles begin to appear within the epithelial lining. These vacuoles increase in number and in size, and coalesce towards the caudal extremity. Figure 7 is taken at the point of transition from the esophagus to the stomach.

---

[1] The photomicrographs were made by Mr. William S. Dunn, Cornell University Medical School, New York.

In the 32-day embryo the originally solid esophagus is fenestrated—except for the extreme anterior extent, about 100 microns, just behind the opening of the larynx—because of numerous vacuoles, which enlarge and coalesce caudally. Figure 8 is taken from the point of transition to the stomach. This portion of the esophagus has the appearance of a meshwork with large irregular areolæ and delicate nucleated trabeculæ (fig. 9). It suggests somewhat notochordal tissue of an embryo of about the 16-day stage. The esophagus is closed medially in a manner similar to earlier stages, 110 microns behind the opening of the trachea (larynx into pharynx). It is completely closed except for minute lateral canals for 100 microns. The stenosed (vacuolated) area extends through 5.33 mm.

In 5 embryos of *Chelydra serpentina* examined the facts are as follows: In a 4.2-mm. embryo the esophagus is open throughout. In a 6-mm. embryo, which corresponds closely in development to the 13-day *Caretta* embryo, the esophagus is solid from a point just behind the origin of the tracheal anlage to its bifurcation, through 670 microns. In another 6 mm. embryo the solid esophagus opens again 240 microns short of the bifurcation of the trachea, extending through only 580 microns. In a 7-mm. embryo the esophagus is solid for 560 microns, from a point 150 microns behind the original tracheal evagination to a point slightly forward of the bifurcation. In another 7-mm. embryo the esophagus is closed from a point 240 microns behind the connection with the trachea to a point 80 microns forward of the bifurcation, through 800 microns. In both the 6 mm. and 7-mm. embryos numerous vacuoles occur in the epithelial lining. Practically identical conditions prevail in *Chrysemys marginata*. In the older embryos the primitive glottis also becomes obliterated through apposition and fusion of its lateral walls.

From the above it appears that after the eleventh day of incubation the esophagus of *Caretta* is solid throughout a greater or less extent until at least the thirty-second day stage. This secondary imperforate area extends from about the point of the connection between the foregut and the trachea to the point of bifurcation of the latter into the bronchi. The closure of the originally open esophagus is accomplished in part by cell-proliferation, chiefly in the dorsal wall of the esophagus, accompanied by a dorso-ventral compression of the cylindric tube into one of rectangular outline; and it progresses in a cephalo-caudal direction. The reestablishment of the lumen begins caudally and extends forwards, the last point of opening being thus at the laryngeal level. The opening is accomplished by a process of vacuolization of the epithelial lining. Judged by the time occupied in the effective vacuolization of the caudal portion of the solid esophagus, it seems reasonable to suppose that the laryngeal end remains closed until, or near, the time of hatching. A matter of cardinal importance concerns the fact that the first point to close in the primitive esophagus is medially at or just behind the level of the tracheal (laryngeal) connection, and the last point to open is just behind this same level. It would seem, on the basis of this observation, as if the object of the transient solidification of the esophagus was to close the opening of the trachea during the greater period of the embryonic development of the lung against the mid- and hind-gut as if to protect the lung against materials contained in the gut-tube, chiefly yolk-globules.

It remains to describe in detail the process of closure and the subsequent vacuolization of the atresia whereby the lumen becomes reestablished. For this purpose embryos of the eleventh, twelfth, sixteenth, and thirty-second days will be chiefly employed. In the light of these additional details the hypothesis above suggested, namely, that the solid esophagus in the turtle embryo has its meaning in relation to the developing lung, perhaps protecting it against the entrance of yolk and other material from the gut, will be discussed; and an attempt will be made to correlate conditions in the turtle with those described for other vertebrate forms, and to interpret the occasional esophageal stenosis in mammalian embryos in terms of a reptilian ancestry. Exactly the same series of stages, as indicated by the histologic structure, occurs in the esophagus of the 32-day embryo in a cephalo-caudal direction, as appears in the greater extent of the esophagus of embryos of successive stages of development as represented in the 11- to 25-day stages.

The esophagus of the 11-day embryo, as stated above, is open throughout. But the lumen has become considerably narrowed by reason of an increase in the number of layers of the lining-cells. The number of layers dorsally varies from seven to nine, cell boundaries being indistinct. The two outermost and the two innermost layers consist of cylindrical cells; the remaining layers comprise chiefly polyhedral and spheroidal cells. Division, both mitotic and apparently amitotic, is most active among the cells of these intermediate layers. The number of cell-layers ventrally and lining the laryngotracheal groove, as indicated by the number of layers of nuclei, is four; but this portion of the epithelium is apparently for the most part a pseudo-stratified columnar type. Mitotic figures are most numerous among the central layers of nuclei—that is, next the lumen.

Where the wide pharynx passes into the oral end of the esophagus the lumen becomes triangular in cross-section, the base directed dorsally. The esophagus at this level is lined by 5 to 6 layers of cells throughout. The peripheral and central cells are cylindrical in shape, those of the intermediate layers have a polyhedral form, and many of the latter are in mitosis. At a slightly lower level the dorsal portion of the lining epithelium thickens, while in the ventral portion, *i. e.*, along the tracheal evagination, the epithelium becomes somewhat thinned. The shape of the lumen at this level, including here in continuity a portion belonging to the esophagus proper and a portion belonging to the laryngo-tracheal groove, is oval in transection, flattened from side to side.

At the level of bifurcation of the trachea the lumen of the esophagus has a narrow, spindle-shaped outline, compressed from side to side. This lumen is lined by a pseudo-stratified columnar epithelium, containing 4 rows of nuclei dorsally and 2 to 3 ventrally. The respiratory anlage, of rectangular outline, flattened dorso-ventrally, is lined by a similar type of epithelium with about 3 rows of nuclei. Mitosis is active among the cells immediately surrounding the lumen. Terminal bars are conspicuous, especially in the tracheal anlage.

The ventral laryngo-tracheal groove becomes separated from the primitive esophagus, and is consequently converted into a tube, by a process involving the approximation and ultimate fusion of bilateral mesenchymal plates, which process extends in a caudo-oral direction. In the area of the advancing residual connection between the separating tracheal and esophageal tubes, *i. e.*, at the level of constriction preceding the separation, the cells are evidently under considerable pressure. The nuclei are in general much smaller, of very variable size, and greatly crowded. They are spheroidal in outline and many show signs of degeneration. Many of these nuclei are vesicular and practically achromatic, except for a relatively large and very chromatic nucleolus. None of these cells are in mitosis, but many of the nuclei are at some phase of amitotic division. In the surrounding mesenchyme, however, especially in the plates which invaginate the esophageo-tracheal tube in the process of separation, mitosis is very active. Along the line of separation, the nuclei in the ventro-medial epithelium of the esophagus and in the dorso-medial epithelium of the trachea are much smaller, more nearly spherical, and more crowded than elsewhere in these tubes. A few smaller spheroidal yolk-globules are present in the lining-cells of both the esophagus and trachea at these stages, but they are relatively more numerous in the esophageal epithelium.

In the 12-day embryo the esophagus practically lacks a lumen (fig. 3). At the level just behind the point of connection between the primitive esophagus and the trachea (definitive pharynx and larynx) the narrow circular lumen of the trachea is lined with 3 to 4 layers of cells, some of which, especially centrally, are in mitosis. The esophagus, just cephalad of this point (*i. e.*, in the region of the glottis) has a T-shaped lumen, lined dorsally with about 9 layers of cells, of cylindric form peripherally and centrally and of polyhedral form in the intermediate layers. Viewed as a whole—*i. e.*, the lumen and its cells—the esophagus is still more distinctly T-shaped, the cross-bar in the esophagus proper being about equal to or even greater in length than the stem or the laryngeal anlage.

At the level here illustrated, figure 3, the minute central opening of the esophagus is surrounded by only 3 to 4 layers of cells, as indicated by the rows of nuclei. By the thirteenth day even this capillary lumen is obliterated and the esophagus is completely closed for about 0.5 mm. This closure is apparently effected by a process of lateral traction (or possibly dorso-ventral compression) upon the originally cylindric tube, causing thus a change in shape of the lumen from an oval or a circular to a horizontal cleft form. This mechanical factor is assisted by the larger number of cell-layers dorsally, the result of greater proliferative activity, and accomplishes finally a close apposition and ultimate fusion of the dorsal and ventral walls. One small lateral vacuole was seen in the epithelial lining of the stenosed esophagus at the 12-day stage; at the 13-day stage three lateral vacuoles had appeared.

The central area of the closed esophagus of this stage consists of irregular stellate cells, resembling a compact mesenchymal reticulum. These cells come chiefly from the dorsal thicker wall, and represent modification products of originally cylindric cells.

At the 16-day stage of development the esophagus is closed for approximately 1.5 mm. At the most anterior point of occlusion the pharynx is wide and somewhat crescentic, with the concavity directed ventrally. The occluded area at this level occupies only the smaller medial portion of the wide pharynx at the point where it passes into the esophagus. The larynx at this level has an oval, slit-like lumen, compressed laterally and lined with a pseudo-stratified columnar epithelium containing 2 to 4 rows of oval nuclei, many of which centrally are in mitosis. The pharynx is lined with columnar epithelium (apparently pseudo-stratified) with 3 rows of nuclei, oval in shape peripherally and centrally. At the most cephalic point of the closure the pharynx remains widely open laterally. Only a few mitotic figures appear among these cells. In the median fused area the central cells are of irregular shape, with spheroidal nuclei, and form a mesenchyme-like syncytium. At this stage the glottis is closed for a short distance, due probably to the pressure exerted upon this portion of the tube by the surrounding mesenchyme in the process of final cephalic division of this portion of the fore-gut into esophagus and trachea. The fusion of the lateral walls of the larnyx and the consequent obliteration of the lumen are probably due in part also to the arytenoid swellings just appearing in this region.

Vacuoles first appear, either singly or in groups, centrally and laterally. These are the areas where the fusion-reticulum is originally less dense, because here the original lumen persisted longest. As the vacuoles form, the nuclei of the syncytium become disposed in the manner of an epithelium around these spaces, as if rearranged under pressure from within the vacuoles. The original smaller vacuoles grow in size and coalesce with adjacent vacuoles. Only very rarely is a mitotic figure seen among the central cells. Mitosis is slight at this stage, also among the peripheral cells. An extremely small, practically negligible, amount of cell-degeneration occurs among the central cells. The vacuoles tend to become confluent in a lateral direction; they are apparently empty and contain no coagulum in stained sections. Their spheroidal shape and the manner of the arrangement of the enveloping cells leave no room for doubt, however, that they were formed, at least in large part, under the influence of a fluid pressure.

In the 25-day embryo the vacuoles are larger and more numerous, and they increase in number and size caudally, where a fenestrated condition of the atretic lumen has become established.

Coincident with this fenestration, the enveloping mesenchyme (tela submucosa) of the esophagus has become looser and more vascular, thus permitting more readily an expansion of the tube under the pressure produced internally during the formation of the vacuoles. This expansion is assisted also no doubt by the growth, as indicated by extensive mitotic activity, of the peripheral portion of the wall, and possibly in part also by external traction exerted by the growing mesenchyme.

At the 32-day stage the lumen of the esophagus is in the fenestrated condition throughout its greater extent, only the most cephalic portion remaining practically solid. The lumen appears to be spanned across by delicate nucleated septa with lateral anastomoses. Where the septa join the main wall of the lumen they spread into triangular multinucleated bases. There is no sign of degeneration among the nuclei of these septa—in fact, a number of the nuclei may be in process of either mitotic or amitotic division. The trabeculæ are subsequently simply drawn into the peripheral portions of the wall and gradually incorporated among the entodermal cells of the lining epithelium.

DISCUSSION.

A complete discussion of the phenomenon of esophageal atresia here described must take into special consideration the following facts:

(1) The inception of the atresia at the 12-day stage is coincident with the early stages of the formation of the respiratory anlages.

(2) The initial point of stenosis is spatially very closely related to the orifice of the developing laryngo-tracheal tube.

(3) The transient solidification of the esophagus progresses in a caudal direction, while the reestablishment of patency progresses in the opposite direction.

(4) The early stenosis of the esophagus, from the eleventh to the twelfth day, results largely from an alteration in the shape of the esophagus, from a tube approximately circular in cross-section to one flattened dorso-ventrally into a rectangular structure with at first a slit-like lumen and later a minute central circular aperture; the stenosis becomes an atresia at the thirteenth day through a fusion of the dorsal and ventral walls, the cells involved becoming converted into a mesenchyme-like syncytium; this process is assisted by the increase in the number of cell-layers dorsally, where mitotic activity is extensive; the factors involved in the change of shape of the esophagus are largely mechanical, incidental to inherent and extraneous tissue growth, the chief element in forcing the growth of the esophageal tube into an adaptive rectangular form being most probably the denser bilateral mesenchymal plates by whose medial approximation and fusion the original esophageo-respiratory anlage becomes converted into the esophageal and tracheal tubes.

(5) The esophageal atresia involves also a transient stenosis and atresia of the oral end (glottis) of the respiratory anlage.

(6) The definitive perforate esophagus becomes established by a process involving the transformation of certain ''intercellular'' spaces in the central syncytium into spherical vacuoles, and the enlargement and confluence of these to form larger irregular areolæ; at this stage (32-day) the relatively wide esophageal lumen is spanned by delicate nucleated septa; these septa are ultimately drawn into the expanding peripheral wall and here incorporated with practically no tissue degeneration; the earlier spherical shape of the vacuoles and the disposition of the nuclei around these spaces indicate their formation under the influence of an internal fluid pressure, but no coagulum appears in fixed and stained tissues; the processes of later vacuolization and incorporation of the intra-luminal septa by the peripheral wall are probably largely the results of the growth expansion of the esophageal tube resulting from active proliferation of the lining-cells and assisted by the now very loose and vascular enveloping mesenchyme, the primitive submucosa of the esophagus.

(7) The lumen of the esophagus is still occluded just behind the laryngeal orifice at the 32-day stage and no doubt remains stenosed until near the close of the incubation period (56 days).

(8) All forms in which an extensive atresia of the esophagus has been reported (elasmobranchii, bony fishes, amphibians, certain reptiles, chick) have the gut open to a huge yolk-sac during the greater portion of the incubation period.

The above-enumerated facts would seem to show conclusively that in the *Caretta* embryo the temporary atresia of the esophagus is a normal phase of the developmental process. This is probably true also of all forms with large yolk-sacs. The occasional esophageal constrictions, stenoses, and atresias in the pig embryo, other mammalian embryos, and in man (a normal phenomenon according to Kreuter, abnormal according to Lewis) probably have their explanation in terms of their reptilian ancestry. These conditions in mammals are apparently very variable and any discussion as to their normality or abnormality in these embryos seems of small value. Congenital stenoses or atresias of the esophagus in humans, as Kreuter has already suggested, represent most likely a persistence or exaggeration of a normal phase of embryonic development.

A suggestion of the teleological significance of this temporary atresia of the esophagus in *Caretta* may be obtained by seeking to disclose what is actually accomplished by the phenomenon. Obviously, it closes the respiratory anlage against the more solid contents of the gut during the greater portion of the incubation period. That the relation of the obliteration of the lumen of the esophagus is close to the development of the respiratory anlage is strongly indicated by the following facts:

(1) Its first appearance about the time the laryngo-tracheal groove begins to be separated from the esophagus and at or just behind the later orifice of the larynx.

(2) The extension of the atresia even into the glottis.

(3) The persistence of the atresia orally until nearly the end of the incubation period, when the lung is already greatly developed, and the yolk is almost entirely digested.

It is difficult to avoid the inference that the temporary solidification of the esophagus is a device to protect the developing lung against the invasion from the gut of yolk-globules. The respiratory anlage, being a derivative of the primitive esophagus, is originally lined by an epithelium which retains in part the ability to digest yolk material; but soon after, as it becomes more and more differentiated into the respiratory type of entodermal epithelium, it must more and more lose the capacity for digesting crude yolk material. It seems reasonable to suppose that large quantities of yolk-globules within the pulmonary anlages would seriously interfere with their normal development. That this is actually the correct interpretation of the significance of the solid esophagus in forms with telolecithal, especially meroblastic, eggs could be definitely established only on the basis of experimental evidence; but lacking such, the available facts speak strongly in favor of such a view. In forms with meiolecithal eggs such a protective mechanism against gut-yolk is superfluous; and it is apparently lacking except in slight and variable degree. A congenital atretic esophagus in mammals could be explained in terms of the persistence or exaggeration of a normal or anomalous embryonic condition, the ontogenetic expression of a phylogenetic experience.

As already described, the obliteration of the esophageal lumen (contrary to Kreuter) is only partly the result of excessive cell proliferation, this being limited to the dorsal wall. The chief factor is a change in form of the cylindric tube, effected by the growth of the esophagus operating against the confining plates of denser mesenchyme, which brings about the separation of the ventral portion of the original esophageo-tracheal anlage to form the laryngo-tracheal tube. The thickened dorsal and the ventral walls of the esophagus are thus brought into apposition and finally fuse, thus obliterating the lumen by a central syncytium.

Contrary to what one might expect *a priori*, the lumen of the esophagus is reestablished without any tissue degeneration. This statement is in agreement with the observations both of Kreuter and of Lewis for human embryos. Lewis believes that the constricted lumen of the esophagus in the human embryo becomes enlarged by a process involving a shifting of mitotic activity from the central layer of cells to the peripheral layers, with vacuoles forming as incidental intermediate phenomena. In the *Caretta* embryo there appears no striking evidence to indicate that such a process enters as a large factor. It is true that in the stages preceding the atresia mitotic figures are most numerous centrally, and again that during the reopening of the lumen mitosis is extensive among the peripheral cells; but mitosis is by no means limited to these regions, and the relatively slight excess in one region or the other at the different periods seems to me quite insufficient to account for the early closure and the later vacuolization of the esophagus in the *Caretta* embryo.

The chief factor in the closing process is the change in shape of the lumen, which brings the dorsal and ventral walls into contact and results in a fusion. And the main factor in producing the early vacuolization would seem to be the collection of fluid in the "intercellular" spaces of the central syncytial mass. The earlier vacuoles are uniformly spherical and the cells are arranged about them in the manner of an epithelium, such as would result if drops of fluid grew in size among compacted cells. The irregular condition of the later vacuolization indicates, however, that another factor now becomes chiefly active—namely, one producing traction upon the walls of the vacuoles from without—thus changing them into more or less delicate and anastomosing septa. This factor is no doubt inherent in the growth of the circumference of the esophagus, the mechanism being enabled to exert a maximum expansive effect by reason of the now very loose, wide, and vascular enveloping mesenchyme, the primitive tela submucosa. In agreement with the observations of Kreuter and of Lewis in the case of human embryos, the vacuolization of the esophageal epithelium involves no tissue degeneration in *Caretta*. Occasional nuclei of the septa are in mitosis, and the whole meshwork, including even the most delicate trabeculæ, is ultimately drawn into the peripheral epithelial wall and thus incorporated among the entodermal cells.

SUMMARY.

1. A series of 26 loggerhead-turtle embryos, ranging from the second to the thirty-second day of incubation, were available for this study. Atresia of the esophagus is initiated during the twelfth day of incubation. The observations are made chiefly on the 11, 12, 13, 16, 20, 25, and 32 day stages, and the conclusions are based chiefly on this selected material.

2. During the tenth and eleventh days of incubation the epithelial lining of the oral end of the esophagus (esophageo-respiratory anlage) thickens greatly dorsally, the result of extensive cell proliferation in this region. During the twelfth day the cylindric tube of the esophagus becomes compressed dorso-ventrally, thus bringing the dorsal and ventral epithelial walls in close apposition. Only the minutest central lumen persists in the oral end of the esophagus for a distance of about 0.25 mm. During the thirteenth day the oral end of the esophagus is rectangular in cross-section and completely solid for a distance of about 0.5 mm. The apposed central cells have fused and have formed a plug of tissue, essentially a mesenchyme-like syncytium.

3. The initial point of atresia is *over*, or *just behind*, the orifice of the separating laryngo-tracheal anlage; and its inception is coincident with the earliest stage in the division of the original esophageo-respiratory anlage into an esophageal and a laryngo-tracheal tube. By the sixteenth day the atresia has extended into the orifice of the larynx, due in part perhaps to pressure exerted by the lateral arytenoid swellings.

4. The chief factor in the temporary closure of the originally open esophagus is the change in shape of the esophagus from a tube approximately circular in cross-section to a structure of wide rectangular form with at first a slit-like lumen and finally a minute central aperture. The cause of the change in shape, upon which the obliteration of the

lumen largely depends, is the combination of growth within the esophagus in opposition to the denser lateral mesenchymal plates, by the invasion and medial fusion of which the laryngo-tracheal groove becomes converted into a tube and incidentally separated from the esophagus distally. This process is assisted, as concerns the obliteration of the esophageal lumen, by the active cell proliferation in the dorsal wall of the esophagus.

5. In the 16-day embryo the atresia of the esophagus extends through about 1,500 microns. Beyond the oral end vacuoles begin to form in the lining epithelium. These represent dilated "intercellular" spaces chiefly within the central syncytial plug of tissue. They increase in number, and enlarge caudally, where they become confluent. During succeeding stages this process of vacuolization continues, until at the 32-day stage only the extreme oral end of the esophagus remains closed.

6. Both the closure and the reestablishment of the lumen of the embryonic esophagus involve mechanical as well as growth processes, but are normal for a certain stage of the embryonic development. The closure is not largely dependent upon intrinsic cell-division, and the fenestration process involves no tissue degeneration or resorption. The level of initial closure and the level of final perforation are approximately the same—namely, the laryngeal level of the esophagus.

7. In the process of vacuolization upon which the opening of the temporarily stenosed esophagus depends, the larger spherical vacuoles are drawn into irregular spaces as if through traction exerted from without. This traction no doubt inheres in the growing and expanding periphery of the esophagus. The esophagus now has a fenestrated appearance in section; its lumen is spanned by more or less delicate nucleated septa which may anastomose, giving to the whole the appearance of a wide-meshed syncytium. Ultimately the trabeculæ are drawn into the lining epithelium and their nuclei incorporated among the entodermal cells of the mucous lining.

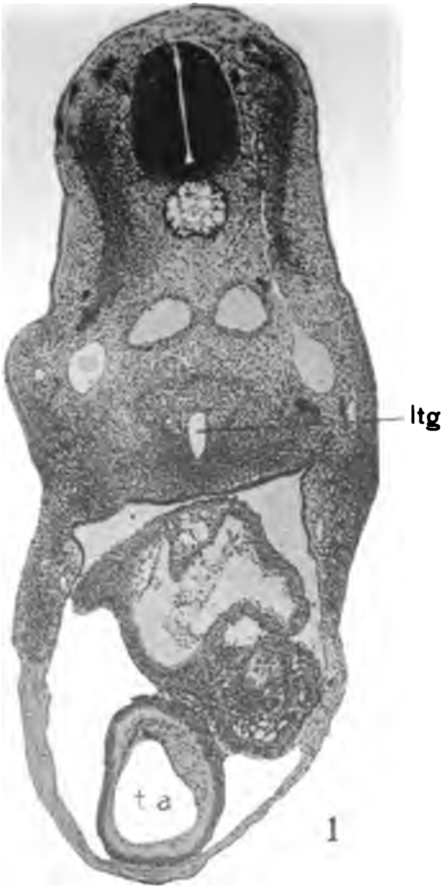
8. The temporary atresia of the esophagus in the *Caretta* embryo would appear to be a device for the protection of the lung during its development against yolk material from the gut, which material could not be digested, but would interfere with normal development of the lung.

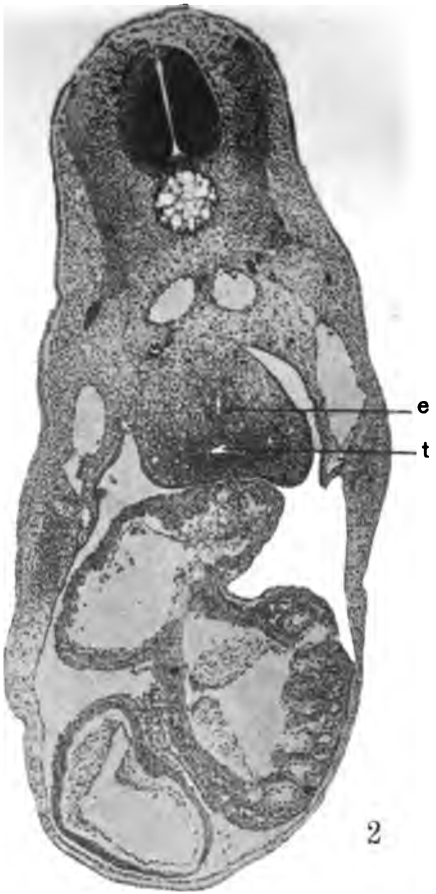
9. This hypothesis can comprehend and correlate conditions in embryos of forms with meroblastic, holoblastic telolecithal, and alecithal eggs. Where yolk is very abundant, as in the meroblastic eggs of fishes, reptiles, and birds, the atresia is relatively extensive and of longer duration; in amphibia the closure is largely of the nature of a stenosis in which yolk-globules are involved, probably in process of digestion, while their forward progress is delayed by reason of the con-

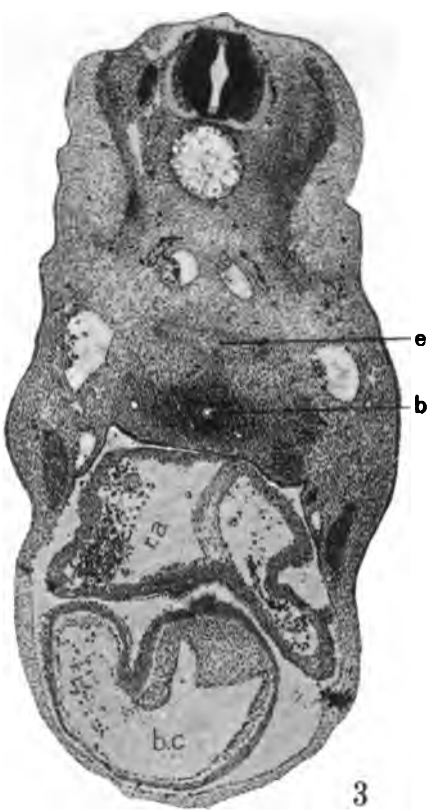
stricted lumen. In most mammals and in man such mechanism is functionally superfluous, and consequently absent except in slight and variable degree. As such it may persist or become accentuated, and produce congenital atresia or stenosis of the esophagus. The relatively frequent embryonic and congenital occlusions of the duodenum just caudal to the pancreatic anlages may perhaps likewise find their explanation in terms of a phylogenetic protective device against yolk invasion.

LITERATURE.

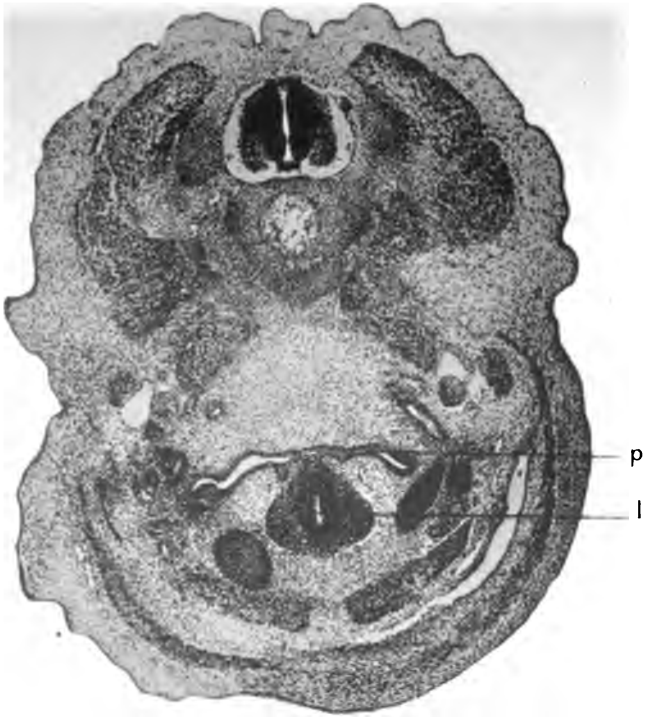
Balfour, Francis M. 1878. A monograph on the development of elasmobranch fishes.
———. 1880. Comparative embryology.
Dean, Bashford. 1896. On the larval development of *Amia calva*. Quart. Jour. Micr. Sci., vol. 38.
Kollmann, J. 1898. Lehrbuch der Entwichlungsgeschichte des Menschen, pp. 340–341.
Kreuter, Erwin. 1903. Solide Oesophagus den Selachier. Diss. Anat. Inst. d. Univ. München. Erlangen.
———. 1905. Die angeborenen Verschliessungen und Verengerungen des Darmkanals in Lichte der Entwicklungsgeschicte. Deutsche Zeitschrift für Chirurgie, Bd. 79, s. 1–89.
Lewis, Frederick T. 1912. The development of the esophagus. Keibel and Mall's "Human Embryology," vol. 2, chap. xvii, pp. 355–368.
Lillie, F. 1908. The development of the chick, p. 312.
Meuron, P. de. 1886. Sur le developpement de l'oesophage. Comptes Rend. hebd. d. seanc. de l'Acad. d. science, T. 102.
Oppel, Albert. 1891. Vergleichung des Entwicklungsgrades der Organe zu verschiedenen Entwicklungszeiten bei Wirbeltieren.



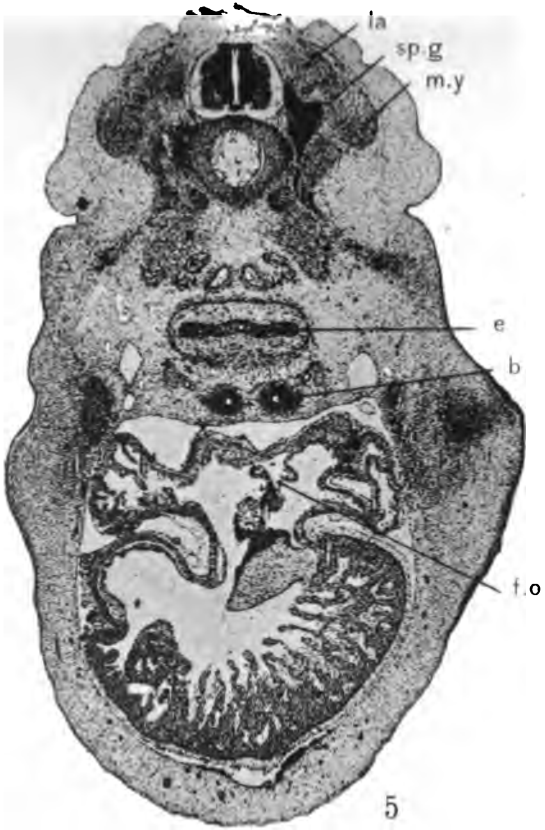




1. Photomicrograph of transverse section of 11-day loggerhead-turtle embryo at level of cephalic extremity of laryngo-tracheal groove (*ltg*). Note the epithelial thickening in dorsal wall of esophagus; (*t. a.*), truncus arteriosus. Just dorsal to esophagus are the dorsal aortae; laterally lie the anterior cardinal (jugular) veins. x 38.
2. Transverse section of same embryo, 150 microns caudad of level shown in fig. 1, at point where trachea (*t*), still connected with the esophagus by an epithelial plate, bifurcates into two primary bronchi. (*e*), esophagus, with patent lumen, and oval outline at this level. The line (*e*) passes across left duct of Cuvier. Photo. x 38.
8. Transverse section of 12-day embryo at approximately same level as that shown in fig. 2. The esophagus (*e*) has suffered compression dorsoventrally and has become rectangular in outline (similar to its more cephalic condition in 11-day embryo); its lumen has become obliterated through fusion of its dorsal and ventral epithelial walls. (*b*) trachea at the point of bifurcation into the bronchi; (*r.a.*) right atrium; (*b.c.*) bulbus cordis. Photo. x 38.



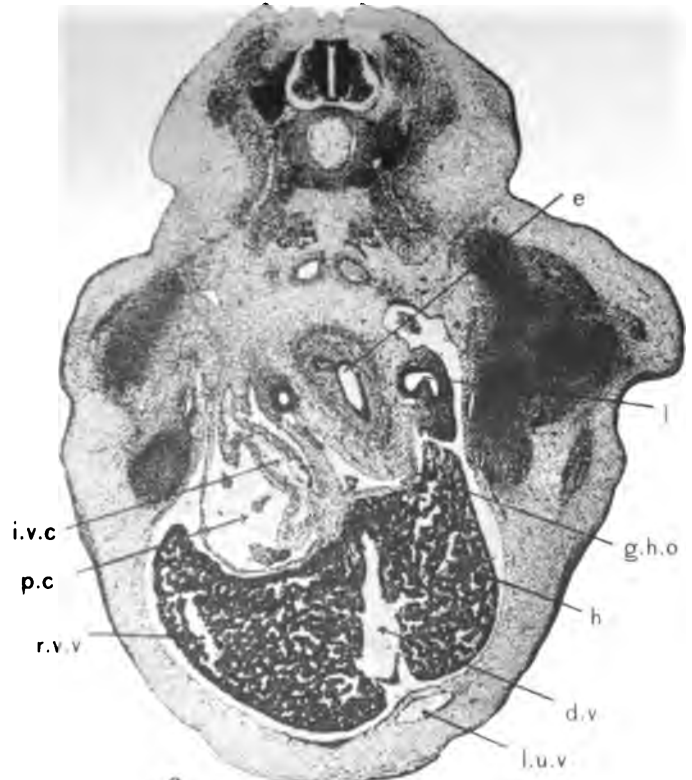
4



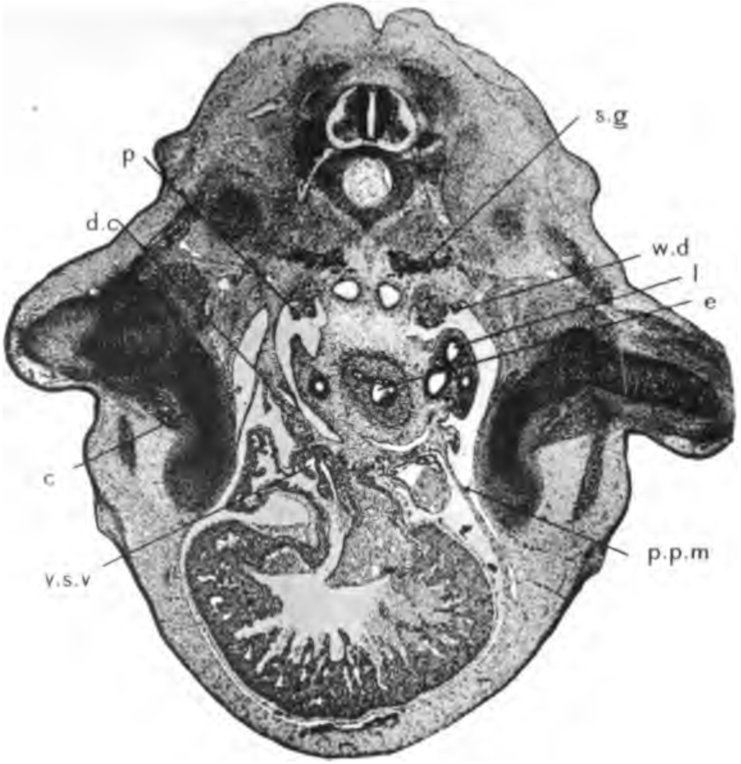
5

4. Transverse section of 16-day embryo at level of transition from pharynx (*p*) to esophagus, showing most anterior point of medial fusion of walls of esophagus. Note that the pharyngo-esophageal atresia has extended into the proximal portion of the laryngeal lumen (*l*) or glottis. The mesenchymal swellings on either side of glottis are the arytenoid anlages. The initial point of closure of the esophagus is at level of laryngeal opening. This is also the level where patency is last reestablished towards end of the incubation period. Photo. x 38.

5 Transverse section of the same embryo, 300 microns caudad of section fig. 4 The esophagus (*e*) has here a rectangular form in section greatly flattened dorsoventrally. The periepithelial mesenchyme (anlage of tela submucosa) has become much looser and more vascular and a more peripheral muscle-layer is beginning to form The esophagus is solid except for a few small disconnected vacuoles, among the entodermal lining-cells. These vacuoles are dilated intercellular spaces. (*b*), primary bronchus. (*f. o.*), foramen ovale. (*l a.*), lamina of avertebra (cartilaginous stage): (*m.y.*), myotome; (*sp. g.*), spinal ganglion. Photo. x 27.



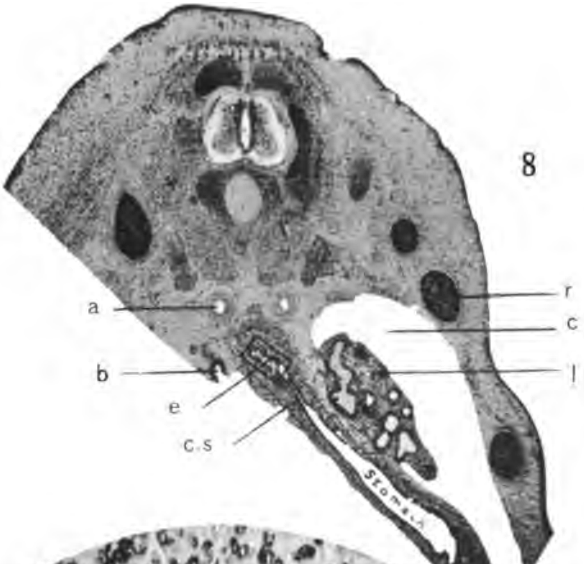
6

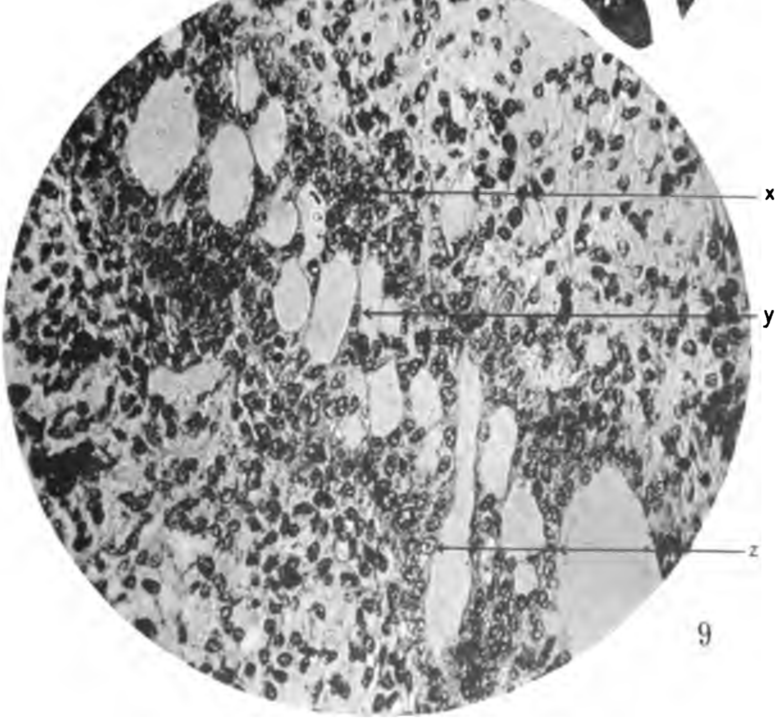


7

6 Transverse section of same 16-day embryo at level of transition of esophagus to stomach (*e*), 1200 microns caudad of section fig. 5. Vacuoles may be seen in the atretic esophagus caudally and in lining of the cardiac extremity of the stomach. (*l*), left lung; (*g. h. o.*), gastro-hepatic omentum, (*h*), liver; (*d. v.*), ductus venosus; (*l. u. v.*), left umbilical vein; (*i. v. c.*), inferior vena cava; (*p. c.*), pericardial cœlom; (*r. v. v.*), right vitelline (omphalomesenteric; hepatic) vein. Photo. x 27.

7. Transverse section of 25-day embryo at level of transition from esophagus to cardiac end of stomach (*e*). The lumen of the esophagus is beginning to be reestablished caudally by a process of vacuolization and a fusion of the vacuoles, without sign of tissue degeneration. The loose and vascular character of the enveloping mesenchyme permits the dilatation of the esophagus necessitated by this process. (*s.g.*), sympathetic ganglion; (*w. d.*), cephalic extremity of fold lodging the Wolffian duct; (*l.*), left lung; (*p. p. m.*), pleuro-pericardial membrane: (*p.*), pronephros; (*d. c.*), right duct of Cuvier; (*c*), cœlom (dorsal parietal recess); (*v. s. v.*), venous valves guarding the opening of right cornu of sinus venosus into the right atrium. Photo. x 20.





8. Sinistro-dorsal portion of transverse section of 32-day embryo at level of transition from esophagus (*e*) to the stomach. The apparent obliquity of the section is due to the sinistral rotation of the cardiac end of stomach (*c. s*). The approximately transverse position of stomach at these stages accounts for its being cut longitudinally in a transverse section of the embryo. Note the extensively fenestrated condition of the caudal portion of the esophagus (*e*, within the rectangle) at this stage. (*r*), rib; (*c*), pleural cœlom; (*l*), left lung; (*a*), right dorsal aorta; (*b*), right primary bronchus. Photo. x 17.

9. More highly magnified photomicrograph (x 300), of portion of esophagus included within rectangle in preceding figure. (*x*), earlier irregular and several-layered condition of lining epithelium of esophagus during period of fenestration of solid esophagus in the process of reestablishment of patency of its lumen; (*y*), a delicate nucleated septum, in process of reincorporation within the later regular single-layered lining of the open esophagus; (*z*), portion where lumen is lined with the later (transient) single-layered epithelium of prismatic cells.

DEPARTMENT OF MARINE BIOLOGY
OF
THE CARNEGIE INSTITUTION OF WASHINGTON
ALFRED G. MAYER, DIRECTOR

PAPERS
FROM THE DEPARTMENT OF MARINE BIOLOGY
OF THE
CARNEGIE INSTITUTION OF WASHINGTON

VOLUME XII

WASHINGTON, D. C.
PUBLISHED BY THE CARNEGIE INSTITUTION OF WASHINGTON
1918

CARNEGIE INSTITUTION OF WASHINGTON
PUBLICATION NO. 252

PRESS OF GIBSON BROTHERS, INC.
WASHINGTON, D. C.

220367
OCT -1 1918
NYL
·C21
/12
O
.7C214
12

CONTENTS

PAGE.
I. *Some Amphibians and Reptiles from Porto Rico and the Virgin Islands.* By HENRY W. FOWLER. 1 plate and 6 figures 1–15
  Leptodactylus albilabris (Günther) 3
  Eleutherodactylus auriculatus (Cope) 4
  Eleutherodactylus lentus (Cope) 6
  Hemidactylus mabouia (Moreau de Jonnes) 7
  Sphærodactylus grandisquamis Stejneger 7
  Mabuya sloanii (Daudin) 7
  Anolis cuvieri Merrem 7
  Anolis mayeri sp. nov 8
  Anolis gundlachii Peters 9
  Anolis cristatellus Duméril and Bibron 10
  Anolis stratulus Cope 11
  Anolis krugi Peters 11
  Anolis pulchellus Duméril and Bibron 12
  Anolis poncensis Stejneger 12
  Ameiva exul (Cope) 12
  Ameiva wetmorei Stejneger 12
  Amphisbæna cæca Cuvier 14
  Epicrates inornatus (Reinhardt) 14
  Leimadophis stahli Stejneger 14
  Alsophis portoricensis Reinhardt and Lütken 14
  Alsophis antillensis (Schlegel) 14
  Alsophis rufiventris Duméril and Bibron 15
  Pseudemys palustris (Gmelin) 15

II. *Fishes New to the Fauna of Porto Rico, with Descriptions of Eight New Species.* By CHARLES F. SILVESTER. 4 plates 17–24
  Aphthalmichthys mayeri sp. nov 19
  Myrophis longleii sp. nov 20
  Myrichthys kecki sp. nov 20
  Tylosurus notatus (Poey) 21
  Corythroichthys ensenadæ sp. nov 21
  Amia conklini sp. nov 21
  Apogonichthys stellatus Cope 22
  Centropomus pectinatus Poey 22
  Diplodus argenteus (Cuvier and Valenciennes) 22
  Eques pulcher Steindachner 22
  Microspathodon niveatus (Poey) 22
  Microspathoden chrysurus (Cuvier and Valenciennes) 22
  Microspathodon fowleri sp. nov 22
  Bodianus rufus (Linnæus) 23
  Iridio maculipinna (Müller and Troschel) 23
  Pseudomonacanthus amphioxys (Cope) 23
  Sicydium antillarum Ogilvie-Grant 23
  Gobiosoma viridistriatum sp. nov 24
  Gobiesox cerasinus Cope 24
  Alticus macclurei sp. nov 24

PAGE.
III. *Oral Gestation in the Gaff-Topsail Catfish, Felichthys felis.* By E. W. GUDGER. 4 plates.......... 25–52
Narrative.......... 27
Historical Account.......... 29
The Breeding Season.......... 30
Distinction between Sexes.......... 32
Method of Transfer of the Eggs.......... 32
Sex of the Egg Carrier.......... 33
Size of Mouth Cavity.......... 34
Size of Eggs and Number Incubated.......... 35
Size of Young and Number Carried.......... 37
Food and Feeding.......... 39
Feeding and Growth of Young while in Paternal Mouth.......... 42
Length of Period of Gestation.......... 43
The Purpose of Buccal Incubation in the Gaff-Topsail.......... 46
The Origin of the Habit of Oral Gestation.......... 46
Literature Cited.......... 51

IV. *Sphyræna Barracuda; Its Morphology, Habits, and History.* By E. W. GUDGER. 7 plates, 5 text-figures.......... 53–108
General Description.......... 55
Color and Markings.......... 56
Jaws and Teeth.......... 59
Manner of Breathing.......... 70
Internal Organs.......... 70
Habits.......... 74
How the Barracuda may be taken.......... 77
Food and Feeding.......... 79
Parasites.......... 80
Use of the Barracuda as Food and Poisoning resulting therefrom.......... 83
Size.......... 93
The Barracuda Dangerous to Man.......... 94
Habitat.......... 99
Fossil Forms.......... 100
Nomenclature.......... 101
Drawings or Figures of the Barracuda.......... 104
Literature Cited.......... 107

V. *Botanical Ecology of the Dry Tortugas.* By H. H. M. BOWMAN. 6 plates, 7 text-figures.......... 109–138
Geologic Formation.......... 111
Climatic Conditions of the Tortugas.......... 113
General Sketch of the Vegetation of the Tortugas.......... 115
Distribution of Species among the Keys of the Group.......... 118
Loggerhead Key.......... 120
Bird Key.......... 123
Garden Key.......... 125
Bush and Long Keys.......... 128
Sand or Hospital Key.......... 130
Middle Key.......... 131
East Key.......... 131
Special Ecology.......... 132
Marine Ecology.......... 137
Conclusion.......... 138

PAGE.

VI. *The Origin of the Electric Organs in Astroscopus guttatus.* By E. GRACE WHITE. 7 plates, 1 text-figure....139–172
Historical Survey of the Subject.... 142
Observations.... 150
Experimental.... 162
Discussion.... 163
Summary.... 166
Bibliography.... 168
Explanation of Plates.... 171

VII. *Toxic Effects due to High Temperature.* By ALFRED GOLDSBOROUGH MAYER..173–178
Summary.... 178
Literature Cited.... 178

VIII. *Nerve-Conduction in Diluted and in Concentrated Sea-Water.* By ALFRED GOLDSBOROUGH MAYER. 1 text-figure....179–183

IX. *A Study of Respiration in Alcyonaria.* By LEWIS R. CARY....185–191
Experimental.... 187
Discussion.... 189
Relation between Death Temperature and Rate of Metabolism.... 190
Literature Cited.... 191

X. *Migration of Insects to Rebecca Shoal Light-station and the Tortugas Islands, with special reference to Mosquitoes and Flies.* By S. C. BALL....193–212
Means by which Insects may reach Rebecca Shoal Light-house.... 196
Opportunities for Breeding of Mosquitoes at Rebecca Shoal.... 198
Conditions at Rebecca Shoal Light-station just prior to June 26.... 199
Methods of obtaining Insects which visited the station.... 200
Insects taken at Rebecca Shoal between June 26 and July 18, 1917.... 201
Migration of the House-fly.... 207
Occurrence of other Diptera at Rebecca Shoal.... 210
Other Insects noted at Rebecca Shoal.... 210
Evidence that Odonata and Leptidoptera Migrate to Tortugas.... 210
Do Sarcophagidæ breed at Tortugas?.... 211
Summary.... 212
Bibliography.... 212

XI. *On Changes in the Sea and their Relation to Organisms.* By J. F. MCCLENDON. 8 figures....213–259
Methods.... 218
Currents.... 221
Records of all Stations and of a Tank of Sea-water.... 222
Relation of Oxygen Tension and Activity of the Nervous System to Metabolism in Cassiopea.... 235
On the Chemical Precipitation of $CaCO_3$ in Sea-water.... 252
Bibliography.... 259

I.

SOME AMPHIBIANS AND REPTILES FROM PORTO RICO AND THE VIRGIN ISLANDS.

BY HENRY W. FOWLER,
Of the Academy of Natural Sciences of Philadelphia.

One plate, six figures.

SOME AMPHIBIANS AND REPTILES FROM PORTO RICO AND THE VIRGIN ISLANDS.

By Henry W. Fowler.

The animals comprising the above-named groups were largely obtained in Porto Rico during the early summer of 1915 by Mr. Charles F. Silvester, while on the staff of the expedition of the Carnegie Institution of Washington. They form an interesting accession to the Museum of Princeton University. Another collection, obtained in the Virgin Islands in 1876 by Mr. A. D. Brown, has been studied in this connection and included in the present report. It is also the property of Princeton University. I am under obligations to Mr. Silvester for the opportunity to study all this material, as well as for various notes, information, etc., kindly placed at my disposal. A series of specimens has been reserved for the Museum of the Academy of Natural Sciences of Philadelphia.

The herpetology of Porto Rico has been so ably discussed by Dr. Leonhard Stejneger, in his paper of that title, published in 1902, that the present account is intended as merely supplementary.

AMPHIBIA.

LEPTODACTYLIDÆ.

Leptodactylus albilabris (Günther). Figure 1.

Ten from the junction of the Arecibo and Tanama Rivers, Porto Rico, on June 9, 1915. As considerable variation in color is noticeable, 5 examples have been selected as an illustration of the dorsal aspect. Two adults show a strongly contrasted color-pattern above, largely of longitudinal bands, of which the median or vertebral is whitish, edged on each side by a blackish band of variable width in places; these blackish bands largely alike. Sometimes a whitish vertebral band gives off a branch on each side in the front interorbital region, which extends over eyelid. Blackish band on each side of snout, including nostril, to eye, then continued behind to embrace tympanum to shoulder, present in all examples. Side of each lip dusky. Several blackish spots on groin and a few obsolete dusky markings along the side. Upper surface of hind limbs strongly contrasted with blackish crossbars. Other examples show lengthwise bands on back obscure, or as irregular blotches, and along the sides as broken small spots. They often form two large Λ-shaped obscure blotches on back before pelvis. Two dark lateral bands may unite in hind interorbital region to form a connecting dark bar. Some examples have the throat dusted with pale dusky, though in most the entire under surface is uniform whitish.

One metamorphosing example and several tadpoles from Utuado; a small example from the Virgin Islands. The Porto Rican examples were obtained along small streams flowing into the Arecibo River about 5 kilometers south of Arecibo, where the lowlands merge into the hills. Active during day-time.

Eleutherodactylus auriculatus (Cope). Figure 2.

Collected 21 examples from the junction of the Arecibo and Tanama Rivers on June 9. This little amphibian is extremely variable in color. Some individuals are pale or very light, others dark, and some of all ages with a narrow whitish vertebral line. Four extremes of color-variation are represented in the accompanying figures of the dorsal surface.

All the examples with the narrow whitish vertebral line (4 individuals) show it bifurcate and extending out on each side of the hind femoral surface toward the under surface of the knee. The larger of these examples are also

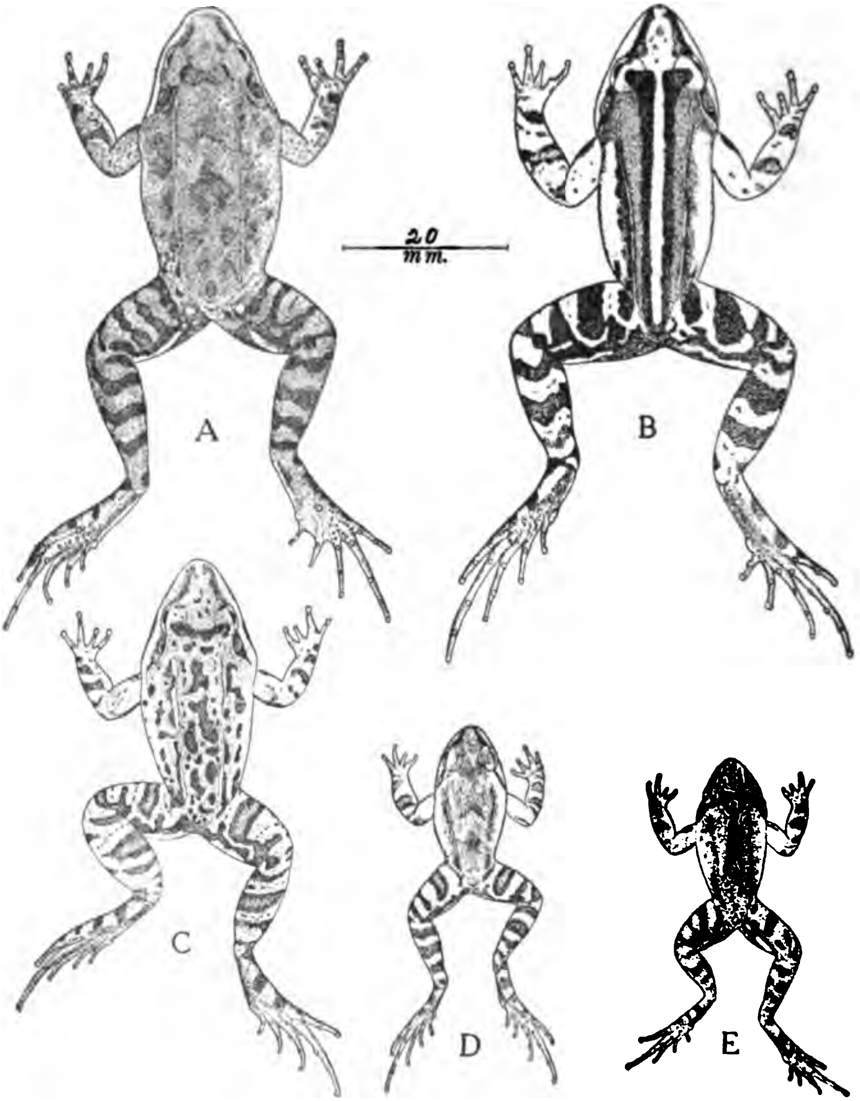


Fig. 1.—*Leptodactylus albilabris* (Günther). A to E, variation in dorsal color—pattern of examples of all ages from Arecibo and Tanama River Junction.

irregularly spotted or blotched with whitish on the back, about the interorbital region, and at each heel. A few spots may also occur elsewhere on the upper surfaces of the tibial regions. The smaller examples have large obscure blotches of slightly darker shade than the general color of the upper surface, which tend to form obscure bars across the tibial and femoral regions. The lower body-surface is similarly variable, from slightly soiled brownish to more or less completely marbled with smutty, in which case there is also a median obscure pale line from the chin to the sternum, with line given off each side to the axillaries.

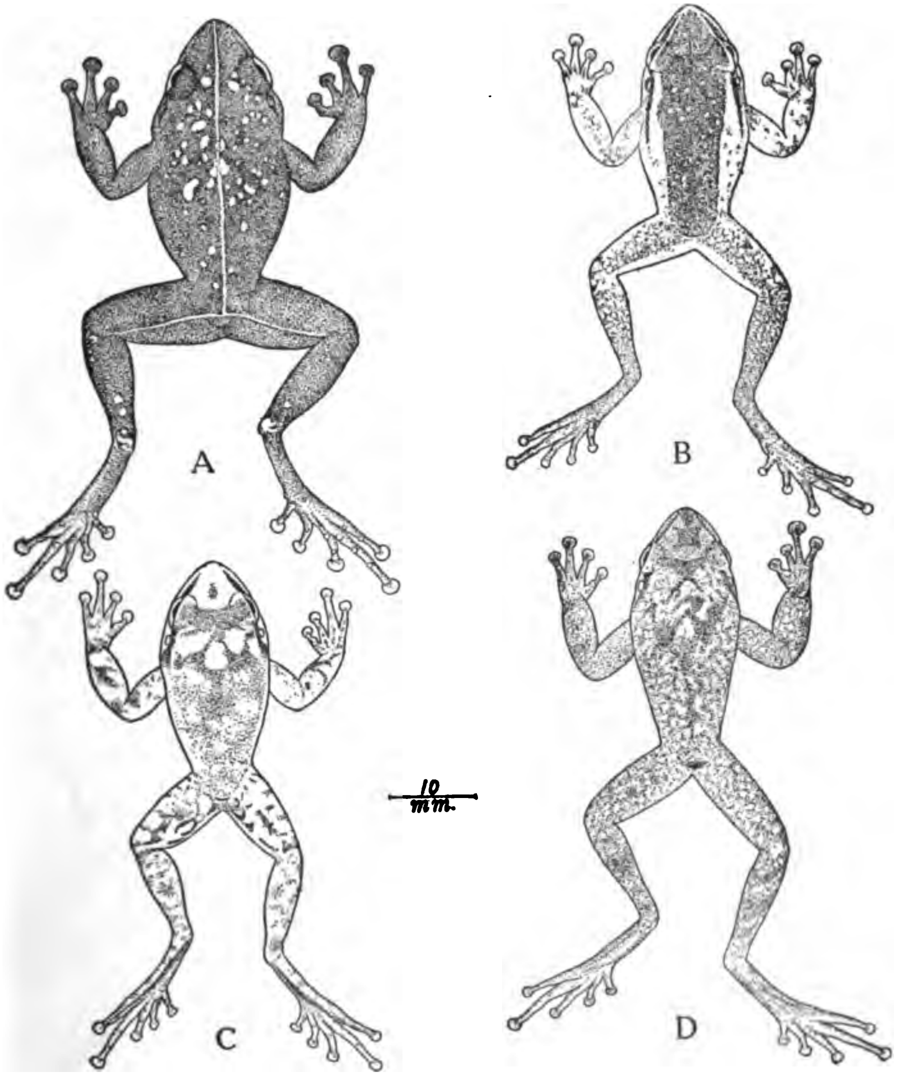


FIG. 2.—*Eleutherodactylus auriculatus* (Cope). A to D, variation in dorsal color—pattern of adult examples from Arecibo and Tanama River Junction.

Four pale examples are also quite variable. One has the front and hind surfaces of the femoral region with rather large dusky vermiculations strongly contrasted with whitish. In this individual there is also a dark-gray interorbital band. Another shows a number of whitish spots on the middle of the back with broad whitish band back from each eye to groin. The lower surface of the body is often uniform whitish.

Large darker olive or brownish examples are more or less uniform in color, though one shows pale areas back from the eye to the groin. They usually have the right and front femoral regions and groin finely speckled brownish on pale ground-color. Most are more or less smutty below.

In a number of small darker examples traces of the light areas back from the eye are seen in two cases and in one of these an accessory short inner line backward and parallel is also present, while the larger bands tend to converge at the middle of the back. All have the limbs above with more or less obsolete dark cross-bars. A dark interorbital band is also usually in evidence.

All examples have the dark line from the eye over the tympanum distinct.

Eleutherodactylus lentus (Cope). Figure 3.

Twenty-one examples from the Virgin Islands. These agree with Meerwarth's figure of *Hylodes lentus*,[1] based on examples from St. Thomas. The

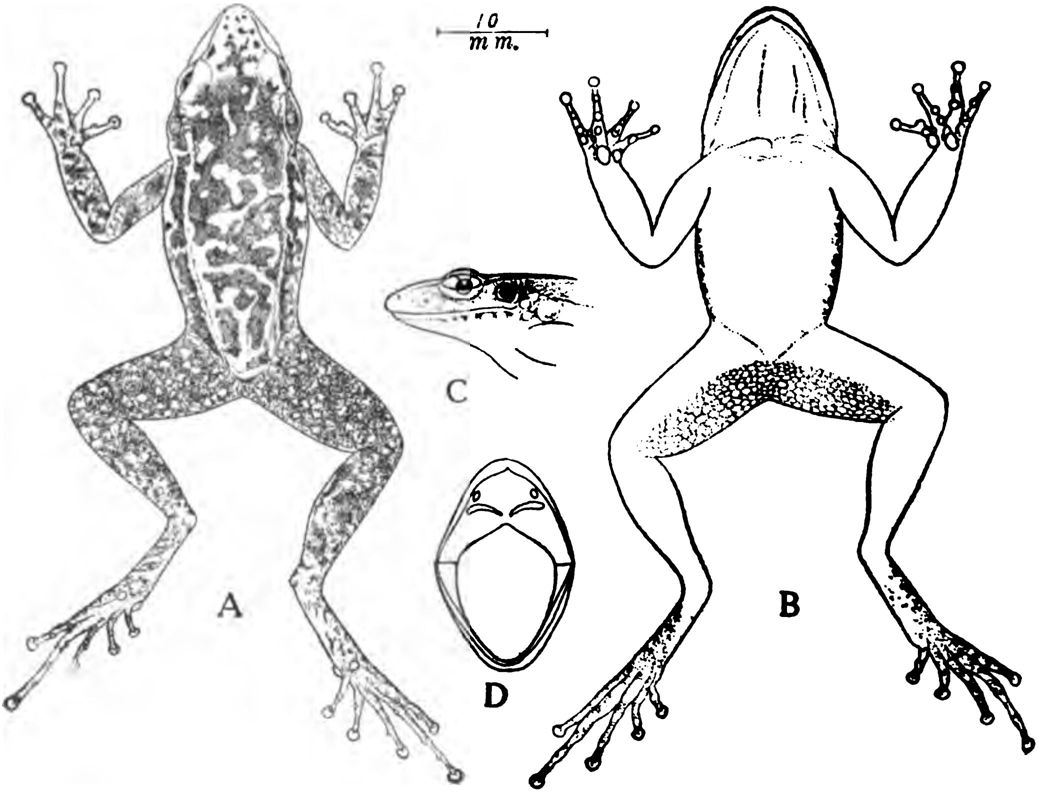


FIG. 3.—*Eleutherodactylus lentus* (Cope). From Virgin Islands. A, dorsal surface. B, ventral surface. C, side view of head. D, open mouth, showing tongue and roof of mouth.

---

[1] Mitth. Naturh. Mus. Hamburg, XVIII, 1901, p. 38, pl. 2, figs. 1, 2.

species appears to be less variable than many of this genus. Although Cope states that the body "is without granulations above, below, or upon the sides," all my examples show them on the lower hind femoral region. As Meerwarth's figure lacks detail I have made the following note. In this species the color-pattern is largely constant. Sometimes the blotches or vermiculations are larger and fewer on the back anteriorly, or they may vary in being finer and more numerous, though the general appearance is similar.

REPTILIA.

GEKKONIDÆ.

Hemidactylus mabouia (Moreau de Jonnes).

Two from the Virgin Islands. According to Dr. Barbour,[1] it was probably introduced into the West Indies.

Sphærodactylus grandisquamis Stejneger.

Three from the Arecibo Road, Porto Rico, about the 70-kilometer post, on June 14; these are similar to Dr. Stejneger's figure,[2] except that the black scapular blotch has in each example two small pure white spots. Two specimens have the dark spots as more or less broken longitudinal bands, though in the remaining example a more speckled appearance is seen and the spots are smaller.

Though only the above examples were secured, this gecko was probably abundant at this locality. They were found under stones and sticks in moist places. Their tails were very brittle and were easily broken off.

SCINCIDÆ.

Mabuya sloanii (Daudin).

Two from the Virgin Islands. One shows at least two pairs of chin shields in contact. Color in alcohol largely grayish olive above, paler or whitish below. From each side of the fronto-nasal plate a brown band extends back, including the upper eyelid, and becomes obscure toward the groin, though on front of body in strong contrast, due largely to darker brownish marginal lines. Another band of similar color extends from the nostrils back, including eye, over ear and shoulder, and fades out on groin. Limbs above with obscure brownish spots. Snout to vent 76 mm., tail 66 mm.

This species is now rare in Porto Rico, a circumstance believed to be due to the mongoose.

IGUANIDÆ.

Anolis cuvieri Merrem.

One from Utuado, 405 mm. in total length. Color in life with iris brownish yellow, a ring of pure yellow next to the black pupil. Scales around eye yellow, barred with black. These colors seem to hold even when the animal changes color from black to green and yellow. In alcohol the color is largely uniform bluish above and paler below. Pale crescent below eye.

Known to the natives as "the devil." They are afraid of this species and believe that it is deadly poisonous. Mr. Silvester informs me it is rare and occurs along water-courses. He saw another example along the main course of the Arecibo River at Utuado, but was unable to capture it.

---

[1] Mem. Mus. Comp. Zool., XLIV, No. 2, 1914, p. 222.

[2] Rep. U. S. Nat. Mus., 1902, p. 605, fig. 52.

Anolis mayeri sp. nov. Figure 4.

Description.—Body long, rather robust, with slight dorso-nuchal fold. Head rather level on top, with broad median depression on prefrontal region. Slight median depression on occipital region. Eyelids granular. Teeth moderately large, and become enlarged posteriorly or below eye, those in front of each jaw small. Dewlap moderate, covered with small imbricated scales. Interorbital slightly convex. Ear-opening circular, deep.

Scales all smooth, rather large. Median or vertebral row of scales down back slightly enlarged, and progressing down sides scales become granular on latter. Very minute granules seen scattered about between scales of back, though not visible to the naked eye and not preventing their imbrication on one or more sides in most cases. Scales on belly larger than most scales on back, and small on under surface of head. Scales on head above rather large and smooth. Supraoculars large, flat, and separated from supraorbital semicircles by narrow, complete row of small scales. Supraorbital semicircles with one or two median plates in contact, and separated from occipital plate by one or two rows of scales. Occipital large, nearly equals ear-opening, and scales around variably large and small. Counting vertically, six rows of loreal scales present, and of more or less uniform size. Six or seven large infraorbitals, last ending well before hind edge of eye. Temporals moderate, and about 12 scales between hind eye-edge and ear.

Limbs rather robust. Scales on upper sides of fore-limbs larger than those on middle of back, slightly keeled, and closely imbricated. Scales along lower front femoral region larger than those on belly, smooth and closely imbricated. Scales on upper surface of femur very small and imbricated. Post-anal plate moderate.

Tail strongly compressed, largely with slight crest. Scales covering sides of tail flat, keeled, those in crest longest and enlarged, a keel forming front edge of each.

Color largely uniform brownish in alcohol, paler below. Some very obscure markings on back, upper surfaces of limbs and tail. Slight pale streak from eye through ear and backward. Another from over shoulder and backward.

Total length 188 mm. (caudal tip damaged) and length from snout tip to vent 67 mm.

Type No. 3151, Princeton University, and 7 paratypes. Virgin Islands.

These examples suggest in many ways that they may be the young of *Anolis cuvieri.* They agree partly in having the scales of the back in places slightly separated from one another by the interpolating of more or less scattered, inconspicuous, and irregular small or minute granules, though these in no way encircle the scales. In general the scales of the back present an imbricated appearance. I have for these reasons allowed the species to stand near *Anolis cuvieri.* Its differences are, however, slight, and as the young of *Anolis cuvieri* is unknown, I first thought it to be such, especially as all my examples are comparatively small. It would appear too great a latitude in variation to admit such characters as these specimens present as simply variations due to age. First is the contact of several pairs of the large scutes of the supraorbital semicircles, rarely separated by a single row of very small scales. Second, the occipital scale is distinct and usually large, though often separated by only one scale from the supraorbital semicircles. The labials are in contact with the infraorbital semicircle, a character constant in every specimen. In agreement with *Anolis cuvieri* are the rows of large scales between the rictus and the ear, which vary from 5 to 8. This character will readily serve to distinguish it from the common *Anolis cristatellus*. The present new form presents a considerable resemblance to *Anolis monensis* Stejneger (known only from Mona Island) in the larger scales and rather uniform coloration, though it has small post-rictal scales like its near ally *Anolis cristatellus*.

Named for Dr. Alfred G. Mayer, of the Carnegie Institution of Washington.

Anolis gundlachii Peters.

Eight examples, the largest 176 mm. in total length, from a small settlement up the Rio Grande, 2 miles above Arecibo, Porto Rico, on June 12, 1915.

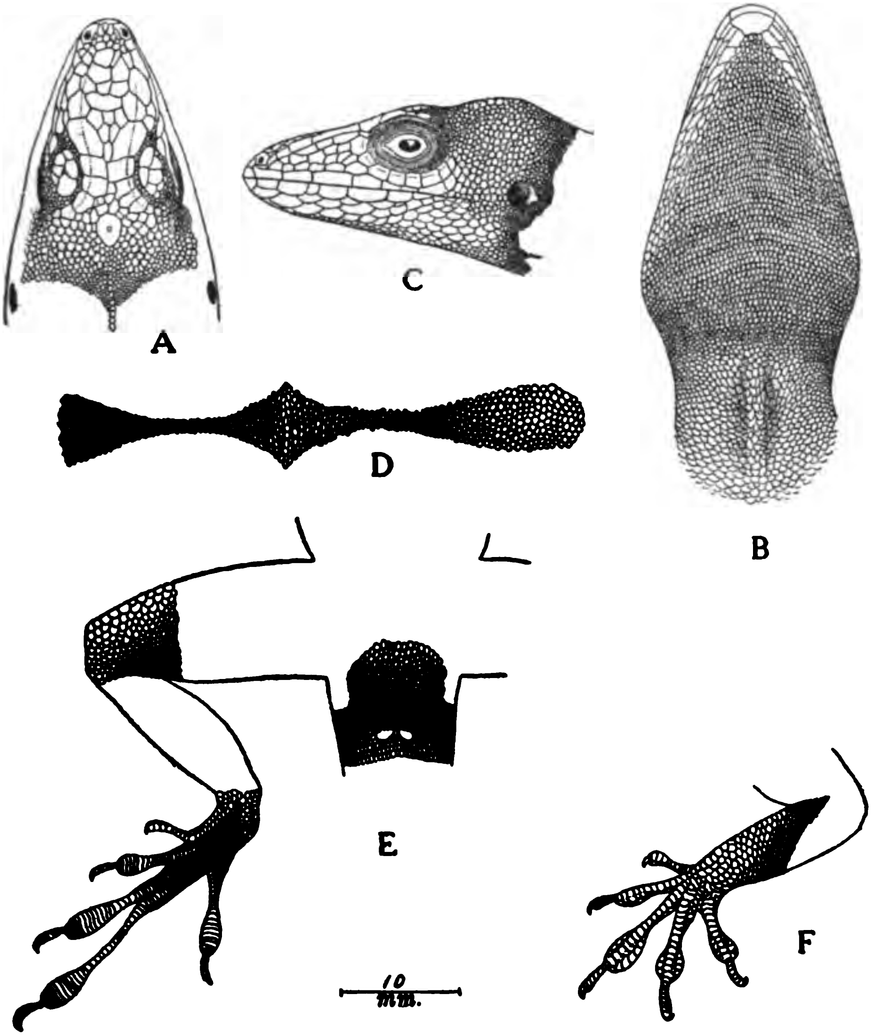


FIG. 4.—*Anolis mayeri* sp. nov.

A, upper surface of head.
B, lower surface of head.
C, side view of head.
D, transverse lepidosis of trunk.
E, lepidosis of hind toes, knee, and vent.
F, lepidosis of fore-arm and fingers.

Color in alcohol uniform dull brownish-olive generally above. Lips pale or whitish, labials obscurely dusted with sooty dots. From gape of mouth back, including ear, whitish band well defined and extending to groin. Immediately below this a gray-brown narrow area all along to groin, fading out in pale color of lower surface, which is largely whitish. Nearly all examples show belly with very pale blue-green tinge. Dewlap yellow-ocher. Some examples are more grayish than others and most show fine specks or dots of dusky on the side above and the costal region below pale lateral streak. The contrasted and bright markings noticed by Dr. Stejneger in living examples are not evident in these preserved specimens. Dark cross-blotches are, however, seen obscurely, though in smaller examples they are far more noticeable. Scales of the supraocular semicircles usually separated by one row of scales at least, often two rows present, and sometimes partly in contact.

Besides the above, 6 examples were obtained from Utuado. These often show but a single row of scales separating the supraocular semicircles.

Anolis cristatellus Duméril and Bibron. Figure 5.

A very large series, represented by nearly 300 specimens from various localities. A large series from Guanica, Porto Rico, shows usually the supraocular semicircles in contact, though the size and disposition of the plates present great irregularity. In color some are very finely vermiculated with darker lines. Often the upper surfaces of the limbs present an obscurely barred

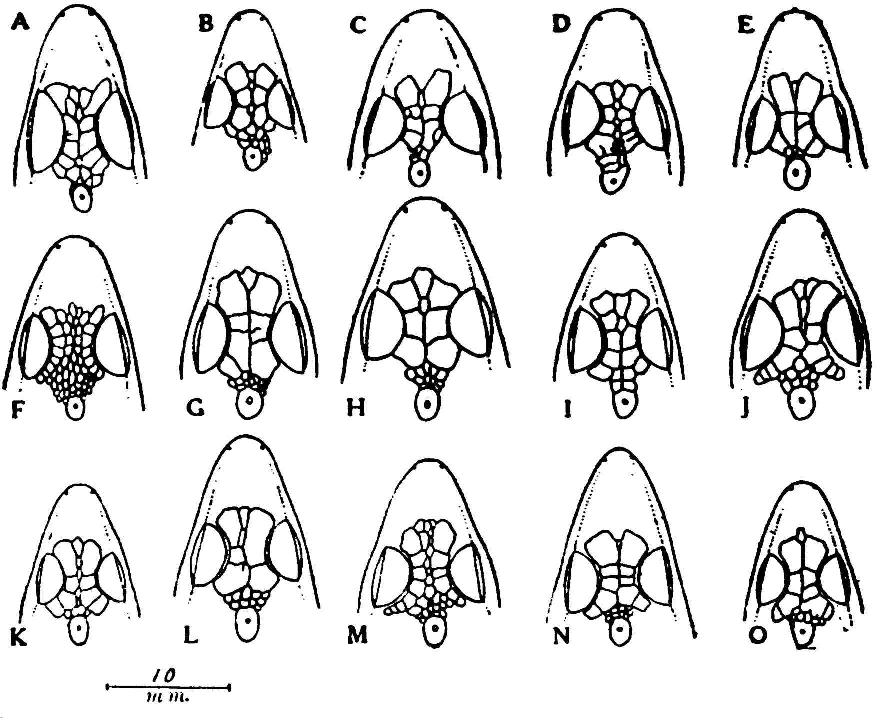


Fig. 5.—*Anolis cristatellus* Duméril and Bibron. Lepidosis of the supraocular semicircles. Figs. A to D and L and M from Guanica, E to H from Arecibo, I to K from Utuado, N and O from Virgin Islands.

appearance, and in some cases they are finely vermiculated. Many examples are more or less olivaceous. In a small series from Guanica one example shows a quite distinct whitish vertebral stripe. Several are pale or light gray.

A large series from Utuado, collected both in the town and down the Arecibo River, June 12, are very variable, both in color and squamation of the head. In color these examples appear much more mottled than others. The chin and throat are variable, and may be streaked or uniform, especially in small or young examples. Some show a considerably developed nuchal crest. Often the labials may be marked with dark blotches, giving the lips a cross-barred appearance. Some examples show five or six obscure dusky or blackish saddles on the back. Occasionally a pale vertebral band is seen down the back. Though the squamation of the head is quite variable, usually the supraocular semicircles have two pairs of plates in contact, often one or two small ones may be interpolated in the median line, and occasionally a median series of small scales (in one instance double) entirely separates them. The largest of this series is 175 mm. in total length.

Thirteen examples obtained at a small settlement up the Rio Grande, 2 miles from Arecibo, on June 14, 1915. Several show a pale vertebral streak on the back and a longitudinal blackish streak along each side of the back, mostly with narrow pale bordering line. Sides of body may also present obscure irregular dark blotches forming smaller spots or speckled appearance below. In some, the lower surface of the body is yellowish in alcohol.

Two from the Arecibo River near the 70-kilometer post, June 14, 1915. One light gray, conspicuously mottled with darker, another with the pale vertebral band bordered each side with a blackish lengthwise band.

A large series from the Virgin Islands, many showing the white vertebral band on the trunk, occasionally interrupted. Many have the chin speckled. Frequently a dark spot occurs behind the occipital plate, sometimes divided to form one on each side.

Anolis stratulus Cope.

Four from Guanica, 2 from Utuado, 3 from a small settlement up the Rio Grande 2 miles from Arecibo, Porto Rico, and 3 from the Virgin Islands. Considerable variation in color is noticeable and a few show distinct dark saddles on the back. All are more or less finely speckled with darker color; in some cases the marbling gives quite a variegated pattern, due to the variable small or large dark spots. The Virgin Islands examples show the back marked with blackish transverse saddles. Dewlap bright yellow in alcoholic specimens.

Variation in the squamation is often seen. Some specimens show one or two pairs of plates of the supraorbital semicircles in contact.

Anolis krugi Peters.

Five examples from Guanica, Porto Rico, now show the dewlap red.

Ten from Utuado, on June 12, show the dewlap crimson in alcohol. Sides and lower surface largely brilliant yellow. In these examples the squamation of the supraocular semicircles is often quite variable. While they may be separated by one row of scales, or sometimes two rows, the rows are frequently incomplete, so that the supraoculars may be in contact with at least one of their pairs.

Five from the Arecibo River near the 70-kilometer post, on June 14. These all have but one row of scales between the supraocular semicircles.

Anolis pulchellus Duméril and Bibron.

Seven very interesting examples from the Virgin Islands which agree very well with Dr. Stejneger's description and figures of Porto Rican material. In alcohol these examples are all pale brownish. A dark brownish, well-defined streak alongside of snout below canthus rostralis to eye and continued back from latter well above ear alongside of back, and on trunk more or less broken into spots irregularly to groin. Below ear on each side of lower surface of head a brownish streak to shoulder, though not evident in young. Upper surface of body or back between dark lateral bands with speckling of brownish, mostly obscured, and arranged largely in lengthwise courses. Similar markings also seen on upper surface of head in some examples.

Variation is seen in the scales separating the supraocular semicircles. In one case a pair of plates of the latter are largely in contact, in another instance two rows of scales intervene, while in the other a single complete row occurs.

Anolis poncensis Stejneger.

Four from Guanica, Porto Rico. This species was heretofore known only from the type locality at Ponce, where it was obtained April 16, 1900. It differs from the other Porto Rican species of *Anolis* in the completely keeled scales of the back, sides, and belly. In coloration my material agrees with Dr. Stejneger's account, the young or smaller example showing the pale vertebral band bordered on each side by a dusky parallel and equally wide band. It also shows a pale obscure orange blotch at and on each side of the occiput, the whole three indistinctly joined. One example is conspicuously greenish above, mottled with dusky.

The squamation of the supraocular semicircles is sometimes quite irregular, and these may have two pairs in contact or be separated by a very narrow median row.

TEIIDÆ.

Ameiva exul (Cope).

Six from Utuado, Porto Rico, some showing the granules extending forward to the second chin-shield. In one an accessory scale is interpolated between the preparietals and in contact with the parietal.

One from the Arecibo River near the 70-kilometer post, on June 14.

One from a small settlement on the Rio Grande 2 miles from Arecibo, on June 14. It shows two very large plates on the inner tibial surfaces.

Among 13 examples from the Virgin Islands, one shows the prefrontals separated by a small accessory scale, and another has the same scale and frontal divided into three equal scales, two of which are in front as a pair; 3 more examples from the Virgin Islands show the occipital plates rounded and subequal; one also shows an additional plate bordering the third and fourth supralabials and interposed between the loreal, first subocular, and preocular.

Ameiva wetmorei Stejneger. Frontispiece and Figure 6.

Description.—Body elongate, rather slender. Tail long, conic. Head conic, muzzle long. Eyelids granular. Teeth rather large, conic, in one row, and becoming larger as they progress backward. Interorbital level. Ear about size of eye, and tympanum not deep. Hind foot about two-thirds combined length of head and trunk.

Front nasal plates meet rather narrowly in contact on top of snout. Nostril in suture between two nasals. Supraocular plates three, in contact with prefrontal, frontal, fronto-parietal, and parietal. Fronto-parietals united as a single plate before 3 large parietals, of which median is much narrowest. Suboculars narrow, first two each half length of last. Loreal in contact with second and third labials, first three suboculars, first supra-preocular, prefrontal, fronto-nasal, and hind nasal. First supraocular in contact with first superciliary, loreal, prefrontal, frontal, and second supraocular. Second and third supraoculars separated from superciliaries by a narrow series of very small scales. Second supraocular in contact with first supraocular, frontal, fronto-parietal, and third supraocular. Third supraocular in contact with second, fronto-parietal, and parietal. Superciliaries of five plates, last small, about equal and all combined only equal in length to anterior one. A row of narrow occipital plates. Six superior and 5 inferior labials to rictus. Ear-opening not denticulated. First pair of chin-shields in contact, second pair separated only in front by narrow single row of small granules. Three rows of scales on gular fold, front and hind rows of about equal width, median row larger. Scales on upper surface of body and limbs minute. Brachium with one series of very broad plates externally and a narrower inner series. Antebrachium with three series of small plates. On under surface of tibia two rows of enlarged plates, outer row of much larger plates, and one above enormously enlarged. Eight rows of abdominal plates. Three large anals, one in front and two behind. Tail covered with rings of smooth scales.

Color in alcohol largely black above, with 7 longitudinal sharply defined narrow lines of very pale blue-green, distributed as follows: Median, much broader than the others, extends from tip of snout to tail, where it broadens

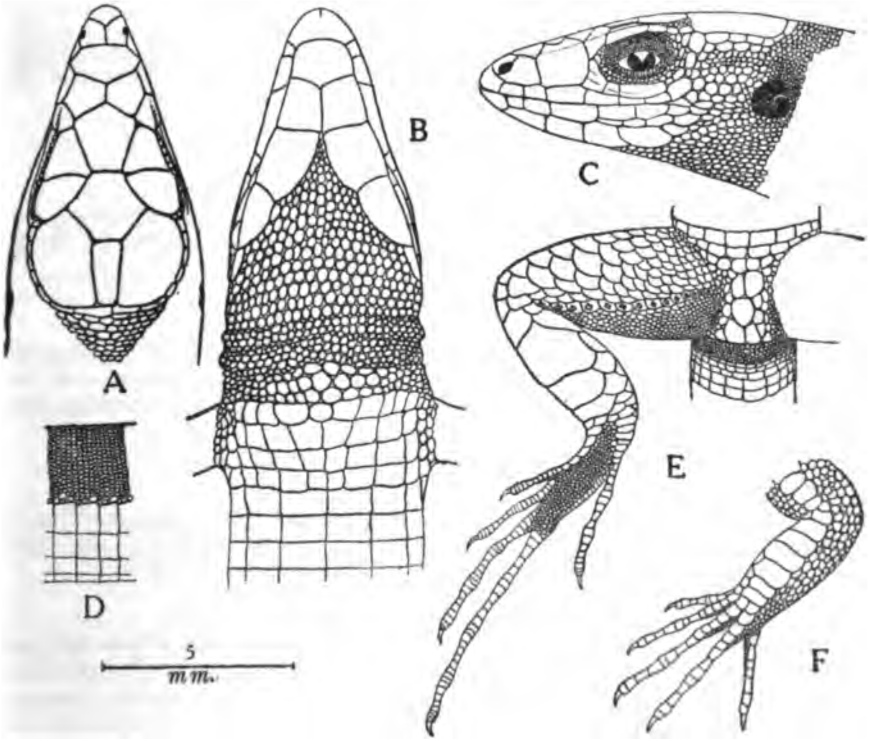


Fig. 6.—*Ameiva wetmorei* Stejneger.

A, upper surface of head.
B, lower surface of head.
C, side view of head.
D, transverse lepidosis of trunk in lateral view.
E, lepidosis of hind limb and ventral region.
F, lepidosis of fore-arm and fingers.

out and, like the others, fades in the more bluish color of the tail. A second streak includes the superciliaries and extends back on upper side of head till above shoulder. On each side, from lower edge of eye back over ear, along its upper edge, a narrow pale blue-green line extends back alongside of tail, fading out behind. Another similar line is on each side from lower ear-edge above shoulder back to groin; below this another still narrower line of same color from axillary to groin. Behind hind leg a single pale blue-green line extends back alongside of tail, fading out behind and much wider than lines ending in groin. Upper surface of limbs blackish like back, each forearm with lengthwise median pale blue-green line, broken in one or more places, and continued up on upper arm as several spots. Several pale spots on outer surfaces of limb in black field. Upper surfaces of hind limb conspicuously spotted with pale blue-green. Under surface of body mostly uniform pale blue-green. Feet and hands dark above, pale below. Tail largely pale bluish, especially terminally.

Color in life with tail greenish-blue (coppery-blue), ground-color above black and below pinkish gray. Dorsal median longitudinal stripe, and three lateral longitudinal stripes, deep straw-yellow. Shorter stripes begin at both nostrils and extend to groin. Legs spotted yellow on brownish-black ground-color.

Total length 103 mm., from snout tip to vent 31 mm.

One example from Ensenada, Porto Rico, June 1, 1915. Collected by Mr. M. H. Sanborn.

The above is the only example I have seen, though several others have been reported. Its striking coloration will at once distinguish it from the well-known *Ameiva exul.* Apparently not reported since described.[1]

AMPHISBÆNIDÆ.

Amphisbæna cæca Cuvier.

One from Guanica. Rings on body 217, on tail 17. Long occipitals more than half length of frontals. Length 130 mm. Collected by Mr. M. H. Sanborn.

BOIDÆ.

Epicrates inornatus (Reinhardt).

One from Guanica, collected by Mr. Sanborn. Ventrals 264, subcaudals 75. Indistinct blotches are seen all along the body, numerous and most distinct on hind part of body and tail. Ventrals largely uniform deep dusky brown. Length 1,675 mm.

CORONELLIDÆ.

Leimadophis stahli Stejneger.

Two from Guanica, besides one obtained on a coffee plantation in the mountains 5 miles west of Utuado. They show ventrals 154 to 157, subcaudals 90 to 92, anals 2, scales transversely on back 19. Length 363 to 382 mm.

Alsophis portoricensis Reinhardt and Lütken.

Two from Guanica, collected by Mr. Sanborn. Ventrals 172 to 180, subcaudals 116 to 118, anals 2, scales transversely over back 17. Length 534 to 1,057 mm.

Alsophis antillensis (Schlegel).

Five from the Virgin Islands. They show ventrals 172 to 183, subcaudals 124 to 130, anals 2, scales transversely over back 19. Length 368 to 943 mm.

---

[1] Proc. Biol. Soc. Washington, xxvi, 1913, pp. 69-72. Guanica.

Alsophis rufiventris Duméril and Bibron.

One from the Virgin Islands, from which the species has not been previously recorded. Ventrals 212, subcaudals 92, anals 2, scales transversely over back 23. Length 943 mm.

EMYDIDÆ.

Pseudemys palustris (Gmelin).

A series of 10 very interesting examples from Guanica Lake, Porto Rico. On first examination they appeared to represent two distinct species, and it appears that two very great extremes of color variation occur in Porto Rico. Dr. Stejneger pointed out in 1902 that "there are indications at hand that there may be some constant differences between those inhabiting the different islands, but the material at my disposal is not sufficient to warrant an attempt to separate them."

Later Dr. Barbour says: "There are specimens at hand from Jamaica (type locality), Cuba, Haiti, and Porto Rico. They do not, however, vary in such a way as to warrant the designation of varieties confined to each island. Stejneger and I (Bull. M. C. Z., 1910, 52, p. 301) had both expected that such subspecies might be defined. As yet, however, the material available for study is very meager. The species is very shy, and is usually confined to lowland muddy pools."

Thus, as little appears to be known concerning this species, a few notes on the variation are given below. All my examples were secured in the lowland lake at Guanica. Dr. Stejneger's figures 185 and 186 represent what may be considered typical (in a restricted sense) of *palustris*. This shows specimens which have the pale lengthwise lines on the sides and lower surface of the head. In this form the carapace is more of a uniform color, without dark blotches or spots, and the plastron may be marked with dark lines along the sutures of the plates, which may vary to very obsolete or even be wanting. About 7 of my examples, representing both sexes of this form, are in the collection.

The remaining three specimens exhibit a different pattern of coloration. They also differ in having the front claws a little longer than the longest of the preceding. The carapace in all is decidedly paler over and about the anterior third than the rest of its extent, showing but few markings, and these now obscure, though the sutures of all the plates are conspicuously blackish. The sutures of the plastron and all the lower marginal plates of the carapace are also marked with blackish. The carapace is also largely speckled or blotched with darker. The color-pattern of the head is strikingly different from the other specimens and is shown everywhere with narrow blackish wavy or vermiculated lines, often broken or irregular. The feet are similar.

The latter variety was first noticed by Gray as *Emys vermiculata*,[1] and the type locality given as tropical America. I have not seen his subsequent plate,[2] though under *Trachemys rugosa* he gives[3] the following description of "*Emys vermiculata* Gray": "Shell 7⅓ in. Back brown, closely black-dotted; sternum and under side of margin very closely spotted. Sternal shields dark edged." The locality is given as the West Indies. Sowerby and Lear figure the *Emys rugosa*,[4] a fairly good representation of the present variety.

The interesting figure by Cocteau[5] agrees with the above in the speckled carapace and dark-edged sutures of the plastron. The head is streaked as in the examples here allowed as typical *palustris*.

---

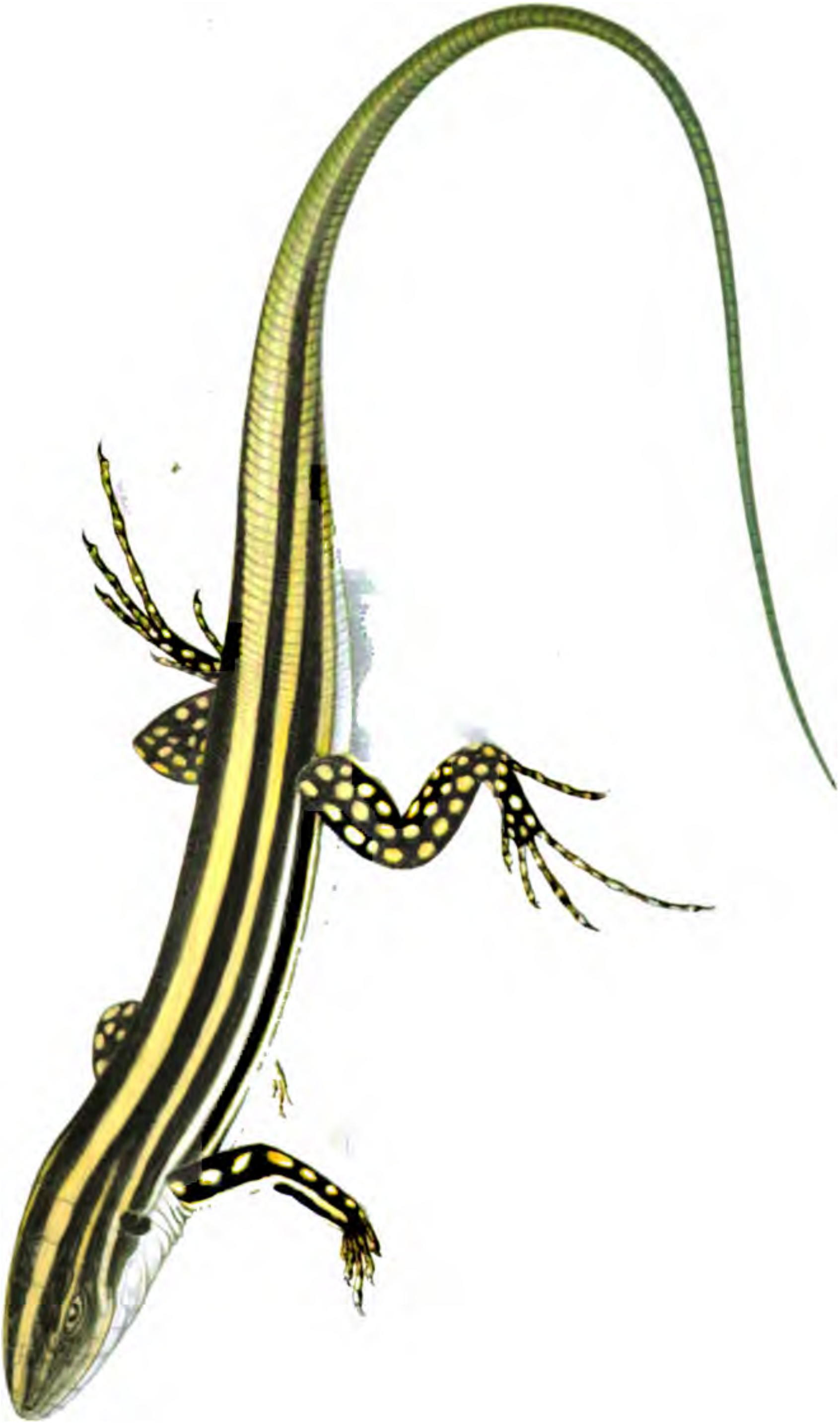
[1] Cat. Tort. Brit. Mus., 1844, p. 25.

[2] Cat. Shield Rep. Brit. Mus., 1855, plate 13.

[3] Hand-List Shield Rept. Brit. Mus., 1876, p. 46.

[4] Tort. Terrap. Turt., 1872, plates 42, 43.

[5] Hist. Nat. Cuba, Sagra, Rept., 1844, plate 2.



Ameiva wetmorei Stejneger. Enlarged about three times.

A HOEN & CO LITH

II.

FISHES NEW TO THE FAUNA OF PORTO RICO, WITH DESCRIPTIONS OF EIGHT NEW SPECIES.

BY CHARLES F. SILVESTER,
Curator of the Zoological Museum of Princeton University.

Four plates.

FISHES NEW TO THE FAUNA OF PORTO RICO, WITH DESCRIPTIONS OF EIGHT NEW SPECIES.

BY CHARLES F. SILVESTER.

Little more than half of the known species of West Indian fishes have been recorded from Porto Rico. Since the work of Evermann and Marsh,[1] Nichols[2] has added 22 species to the fauna of the island. Two of these were new to science.

In May and June 1915, the writer spent three weeks on the island as a member of an expedition to Porto Rico under the auspices of the Carnegie Institution of Washington. The results of this work are to be incorporated with a more extensive investigation of the fishes of the island, but it seems best at this time to publish, at least, descriptions of the new species.

It is with pleasure that the writer acknowledges his indebtedness to Dr. Henry W. Fowler for much valuable assistance and supervision in working up the collections. Through the courtesy of the Academy of Natural Sciences of Philadelphia comparisons have been made with specimens in their collections.

MORINGUIDÆ.

Aphthalmichthys mayeri sp. nov. Plate 1.

Head 9.2 in body; 4.2 in tail; 13.4 in total length. Depth same as upper jaw, 5 in length of head, about 65 in total length; snout 7 in head. Lower jaw projecting; anterior nostril tube-like, near end of snout; posterior nostril larger, in front of eye. Teeth retrorse; eye small but not rudimentary. Interorbital space two-thirds length of snout. Vertebræ 74+46. Spinous processes prominent anteriorly; transverse processes large throughout. Gill-opening one-half the isthmus, which is the same as snout. Gape not as wide as in *A. caribbeus*. All the fins very rudimentary, microscopic, excepting caudal; pectoral less than one-half width of gill-opening; dorsal and anal begin length of head behind vent and continue as very fine lines to end of tail, where they enlarge into the easily visible caudal. Lateral line present and prominent, with series of microscopic, open pores; blood-vessel follows course of and shows through skin on lateral line.

Color in life orange-yellow above; ventral surface slate-blue, merging into whitish in anterior part of tail region; posterior part of tail entirely yellow; iris blue. Tip of lower jaw and area behind eye reddish, fading to pale flesh-color in spirits. Color in *A. caribbeus* much darker in spirit preparations.

In a preliminary list of "Fishes New to the Fauna of Porto Rico," published in Year Book 14 of the Carnegie Institution of Washington, the writer was in error in placing this species in the family Murænesocidæ and in making it the subject of a new genus. As Gill and Smith have shown (Science, n. s., vol. XI, No. 286), the genus *Stilbiscus* is identical with the genus *Moringua* and must be referred to the family Moringuidæ. This family up to that time had not been known to be represented in American waters.

---

[1] Aquatic Resources and Fisheries of Porto Rico, U. S. Fish Commission Bulletin for 1900.

[2] Bulletin of the American Museum of Natural History, vol. XXXIV, article VII, pp. 141–146.

A careful comparison has been made with a specimen of *A. caribbeus* collected at San Juan by Professor Conklin. These two species are very closely related, but *A. mayeri* may be distinguished at once by its striking color. The type was kept alive for more than two weeks in an aquarium at Guanica and during this period no color changes could be noted; in spirits *A. caribbeus* is much darker in color; microscopic examination shows many more chromatophores. The grooves for the dorsal and anal fins, so well marked in *A. caribbeus*, are very little evident in *A. mayeri*.

Two specimens of this beautiful little eel were obtained on the sand flats around mangrove swamp west of Guanica Harbor at very low tide.

Type, No. 3073, Zoological Museum, Princeton University; 310 mm. long.

Paratype, No. 3126, Zoological Museum, Princeton University; 28 cm. long.

Named for Dr. Alfred G. Mayer, director of the expedition.

MYROPHIS.

Myrophis longleii sp. nov. Plate 2, fig. 1.

Head 3.5 in trunk, 5.7 in tail. Depth of body at gill-opening 3.3 in head. Upper jaw projecting; teeth sharp, more or less irregular on maxilla and vomer, indicating two series; series more or less single on mandible. Eyes rather high. Interorbital space 1.5 times eye. Snout broad. Eye 1.5 in breadth of snout between anterior nostrils, 2.5 in snout. Tail compressed. Body slightly compressed. Greatest width of head greater than body behind gill-opening. Vertebræ, abdominal 53, caudal about 100. Ribs developed. No neural spines. Vertical fins well developed; dorsal beginning two-thirds length of head in front of vent, anal beginning directly behind vent; both continuous into caudal, which is wider and completely surrounds end of tail. Anterior nostril tube-like, near end of snout; large pore above this on top of snout. Posterior nostril in upper lip.

Color in life, light olive-green with very fine punctation above, lighter below.

Type, No. 3083, Zoological Museum, Princeton University. Length, 12 cm.

Dug from sand flats west of Guanica Harbor.

Named for Dr. W. H. Longley.

OPHICHTHYIDÆ.

Myrichthys kecki sp. nov. Plate 2, fig. 2.

Head 4 in trunk, 9.5 in total length; eye 3 in snout, snout 4.6 in head. Teeth more or less blunt in single series; vomerine teeth present. Pectoral small, as wide as gill-opening, but very short and rudimentary. Isthmus about 3 times width of gill-opening. Numerous pores on top of head and on lower jaw; large round pore on center of opercle. Anterior nostril near end of snout in large tube which projects downward. Posterior nostril under eye in lip. Dorsal well developed, begins on nape and extends to near tip of tail; anal very low, beginning directly behind vent and extending to near tip of tail. Tail projecting very little beyond dorsal and anal. Branchial chamber rather large.

Color in life light transparent brownish-green, darker above, series of about 20 clusters of darker spots along lateral line, hardly distinguishable in life. This specimen shows different color-markings from other members of this genus, but is no doubt a young specimen and therefore little importance can be laid on the color, which is so subject to change with the growth of the individual. The relative measurements and proportions warrant classifying it as a new species.

One specimen, dug from mud flats near mangrove island west of Guanica Harbor. Length, 7 cm.

Type, No. 3082, Zoological Museum, Princeton University.

Named for Mr. Thomas Keck.

BELONIDÆ.

Tylosurus notatus (Poey).

One specimen, 26 cm. long, seined among mangrove islands west of Guanica Harbor.

No. 3121, Zoological Museum, Princeton University.

SYNGNATHIDÆ.

Corythroichthys ensenadæ sp. nov. Plate 2, fig. 3.

Rings 18+33; dorsal 19, on 1+4 rings; pectoral 12; caudal 10; anal 2. Head 9. Eye 5. Body 1.5 in tail. Cranial ridges strong, median keel on snout to middle of eye; ridge on occiput high, notched in front and behind. Supraocular ridge beginning in tubercle in front of center of eye; two ridges on opercle. Nostril double, in front of and near eye, tubed, dorsal tube short, ventral tube longer. Keels on body and tail strong; lateral keel of body running into ventral caudal keel. Belly convex, keel strong.

Body with 22 yellow and 22 brown bands, each covering from one to two body rings, some of which divide on the ventral surface of belly into rings and blotches of brown. Brown bands edged with darker. Bands on snout become irregular spots and blotches. First brown band crosses interorbital space through eye and obliquely downward and backward to large brown area on ventral surface of opercle.

Closely related to *C. albirostris*, but well defined in color-markings.

Vertebræ and body rings correspond.

Type, No. 3084, Zoological Museum, Princeton University. Length 10.5 cm.

From bunch of coral off Ballenas Point.

Named for a party of friends from Ensenada, who obtained this specimen.

CHEILODIPTERIDÆ.

Amia conklini sp. nov. Plate 3, fig. 1.

Head 2.6; depth 2.7; eye 2.6; interorbital 3.2; dorsal VI–I, 9; anal II, 9; scales 2–27–7; eye large, 2.5 in head; head broad; mouth rather large. Lateral line complete, following curve of back to caudal peduncle, where it dips down to center line. Anterior nostril near front of snout; posterior nostril large oval opening in front of center of eye.

Color orange-red with clusters of small black specks bordering scales over entire body. Caudal peduncle with large oblong black spot; caudal edged with black; spinous dorsal black; soft dorsal and anal with black bar at base; a black bar extending downward and backward on front of preopercle from eye; another bar extending transversely on nape and down on opercle, where it is more or less broken up into series of dots.

Differs from *A. sellicauda* in color markings, larger eye, and more robust body. Examples of *A. sellicauda* were obtained on the same coral reef, and this species differs distinctly from *A. conklini*, the proportions and color markings being quite different. *A. conklini* does not fade to white in spirits and the clusters of minute dark dots bordering each scale do not fade.

Two specimens, 4.5 and 5 cm. long, obtained with dynamite on coral reef off Guanica Harbor.

Type, No. 3080, Zoological Museum, Princeton University. Length, 5 cm.

Paratype, No. 3128, Zoological Museum, Princeton University. 4.5 cm. long.

Named for Professor E. G. Conklin.

Apogonichthys stellatus Cope.

Three specimens, 1.5 to 2.5 cm. long. Inhabits dead sea-urchin shells. Almost every sea-urchin skeleton in small muddy area west of Guanica Harbor was inhabited by one of these delicate little fishes. The individual was never found more than a foot or two away from its shelter and when disturbed or approached darted into the shell. At low tide or when the surf was breaking over the mud flats the fish was invariably found inside the shell.

Measurements of the largest individual are as follows: Depth 3; head 2.7; eye 3; snout 5; scales 2–27–6. The chromatophores take the form of stellations in spirit preparations. In life this fish is a uniform dark brown, almost black, with no evidence of bars or spots.

No. 3048, Zoological Museum, Princeton University.

CENTROPOMIDÆ.

Centropomus pectinatus Poey.

One specimen, about 20 cm. long, from Guanica Lake.

SPARIDÆ.

Diplodus argenteus (Cuvier and Valenciennes).

From coral reef off Guanica Harbor.

SCIÆNIDÆ.

Eques pulcher Steindachner.

One specimen, 5 cm. long, from cluster of coral rock in shallow water west of Guanica Harbor.

No. 3097, Zoological Museum, Princeton University.

POMACENTRIDÆ.

Microspathodon niveatus (Poey). Plate 4, fig. 1.

Head 2.7; 3.4 in total length. Depth 1.7; 2.5 in total. Eye 2.5; snout 3. Dorsal XII, 15; anal II, 13. Scales 3–25+6–10. Mouth small; maxillary reaching to nostril. Upper teeth small, movable; lower teeth twice as large and more or less fixed.

Color in life uniform deep marine-blue with about 35 pure-white silvery spots, each the size of a scale, covering the entire body. More numerous on dorsal fin and dorsal part of head and body. When seen in the sunlight these spots reflect light and appear like so many points of fire.

Many specimens of this beautiful fish were seen swimming among the corals of the western end of Rajos de la Parda; they varied in length from about 2 to 4 inches. The color was the same in all. Only one specimen was obtained, 2.12 inches long, from which the above description was taken.

No. 3099, Zoological Museum, Princeton University.

Microspathodon chrysurus (Cuvier and Valenciennes).

Several specimens observed on the coral reefs off Guanica Harbor, but none were obtained.

Microspathodon fowleri sp. nov. Plate 4, fig. 2.

Head 3.2; depth 1.87. Dorsal XII, 15. Anal II, 13. Scales 3–29+7–10, 21 pores. Eye 3.9. Body compressed, but rounded; profile from snout to dorsal evenly rounded; slight indication of fleshy crest at nape in 1 individual, not indicated in 4 individuals. No fleshy corrugations on anterior profile in 4 individuals, indicated in 1 individual. Nostril small. A pigmented, fleshy, tongue-like flap in roof of mouth. Upper teeth small, movable, in semicircular series; teeth of lower jaw large and fixed, in a series rounded in front and concave on sides. Length of caudal peduncle about 2.5 in head, its depth 2.2 in head. Dorsal elevated and ending in a pointed tip behind. Color uniform deep-black to uniform bluish-black, with indication of yellowish tinge under scales; fins uniform black.

Type, No. 3060, Zoological Museum, Princeton University. Length, 19 cm. Five individuals, 13 to 19 cm. long, from coral reef off Guanica Harbor.

Named for Henry W. Fowler, in recognition of his contributions to ichthyology.

LABRIDÆ.

Bodianus rufus (Linnæus).

One specimen, about 15 cm. long, from coral reef off Guanica Harbor.

Iridio maculipinna (Müller and Troschel).

Common on the coral reefs off Guanica Harbor.

MONACANTHIDÆ.

Pseudomonacanthus amphioxys (Cope).

Three specimens from coral reef off Ballenas Point.

GOBIIDÆ.

Sicydium antillarum Ogilvie-Grant.

Several specimens, 5 to 12 cm. long, obtained from mountain streams flowing into the Arecibo River in the vicinity of Utuado. Only one specimen corresponds to the description of the type; 12 other specimens of this genus were obtained; these vary enough to indicate 6 different species. However, careful comparisons would indicate that much of this variation is individual and not specific. For example, microscopic examinations indicate that the filamentous character of the spinous dorsal is sexual and not specific. Two male specimens of what appeared to be *S. caguitæ* had the dorsal filaments, while three females did not possess this character. The scales in the 12 individuals varied from 53 to 84. Young individuals show fewer scales than older ones.

Color in life varied from almost pure white to blue black. Mr. M. H. Sanborn invited my attention to examples of this genus which were almost pure white, found in small mountain streams flowing from caves. Several specimens of this beautiful little fish, 2 to 3 inches in length, were observed by the writer and one specimen was obtained. Observations would indicate that this is a breeding female of possibly a new species. The morphological characters, however, do not warrant making a new species until other individuals of both sexes can be obtained, as well as specimens taken at different seasons of the year.

Several individuals showing the color markings of *S. plumieri* were obtained. They do not show the emarginate caudal nor the large number of scales. They are probably the young of *S. antillarum*.

The writer is of the opinion that *S. vincente* Jordan and Evermann is the young of *S. plumieri* (Bloch).

The fin rays and the vertebræ were constant in all specimens.

Dorsal VII, 11. Anal 11. Vertebræ $26 = 10 + 16$.

Gobiosoma viridistriatum sp. nov. Plate 3, fig. 3.

Gobius lineatus Poey, Mem., II, 424, 1861, Cuba. Preoccupied by *Gobius lineatus* Jenyns.

Head 3.4 to base of caudal, 4.2 in total length; depth 5 in total length. Dorsal VII–I, 11; anal 10; ventral 6. Snout same as eye; eye 4 in head; interorbital space about 3.5 in head. Gills 4; pseudobranch present. Nostrils double, well separated. Anterior nostril in tube above upper lip; posterior in very short tube in front of upper third of eye; upper lip large. Branchiostegals 3. Vertebræ 11+16? A few large open pores on head, large one behind eye. A series of very minute barbel-like organs following course of under jaw extending back to ventral surface of opercle.

Body with 20 well-defined vertical brilliant green bars, edged with darker, and separated by 21 narrower yellowish-white bars. A brilliant red band extends from tip of snout through eye to above gill-opening. Bars and bands fade to brown and white in spirits.

Obtained from branches of dead coral east of entrance to Guanica Harbor.

Type, No. 3079, Zoological Museum, Princeton University. Length, 23 mm.

Paratype, 19 mm., No. 3126, Zoological Museum, Princeton University.

This is undoubtedly the species described by Poey under the name of *Gobius lineatus*, from Cuba. It is quite distinct from *Gobiosoma multifasciatum* of Steindachner and for this reason is here described and named.

GOBIESOCIDÆ.

Gobiesox cerasinus Cope.

Quite common around coral reefs in vicinity of Guanica Harbor. Usually hidden in crevices of rocks.

BLENNIIDÆ.

Alticus macclurei sp. nov. Plate 3, fig. 2.

Head 4.2; depth 4.3. Dorsal XII, 20; anal 23. Head slightly longer than deep; anterior profile vertical, slightly concave. Eye 3.25 in head. Pectorals reach to vent or beyond in male, not quite to vent in female. Ventrals 1.5 in head. Upper lip of male large; teeth pectinate; canines large; fringe around upper and lower lips; upper lip pigmented, lower not pigmented in spirit specimens, yellow in life. Supraocular tentacles slender; large tentacle medial to each anterior nostril, which divides into 5 or 6 tentacles; two nuchal tentacles in male, smaller in female. Numerous large pores on head, one group behind and below eye, another on supraopercular region. Dorsal fin entire, extending from nape to caudal; last dorsal ray bound down for two-thirds of its length; last anal ray free. Last dorsal and last anal rays small; do not show except in radiograph.

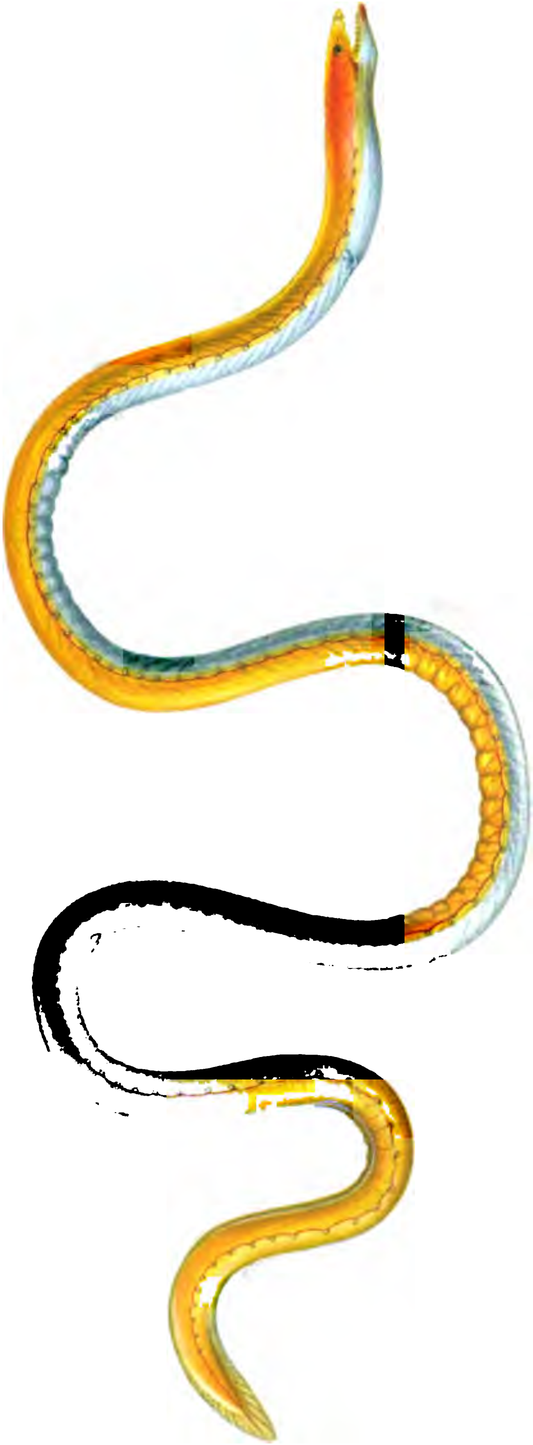
Differs generally from *A. atlanticus* in coloration. General color brownish, livid purplish on costal region; filaments above eyes and edges of lips bright orange. Iris slaty. Dorsal olive-greenish with median dusky lengthwise band anteriorly, contrasting strongly with whitish or pale upper half of fin, but sloping up behind so that edge of dorsal is dusky posteriorly. Upper front edge of dorsal broadly orange below, with submarginal area of whitish. Caudal olivaceous, dusky medially, grayish below, yellowish above; anal dark neutral tint, edge blackish; pectoral pale orange, red on lower half; ventral pale. Vertebræ 12+22.

Two specimens from dead coral reef west of Guanica Harbor.

Type, No. 3081, Zoological Museum, Princeton University; 5.5 cm. long.

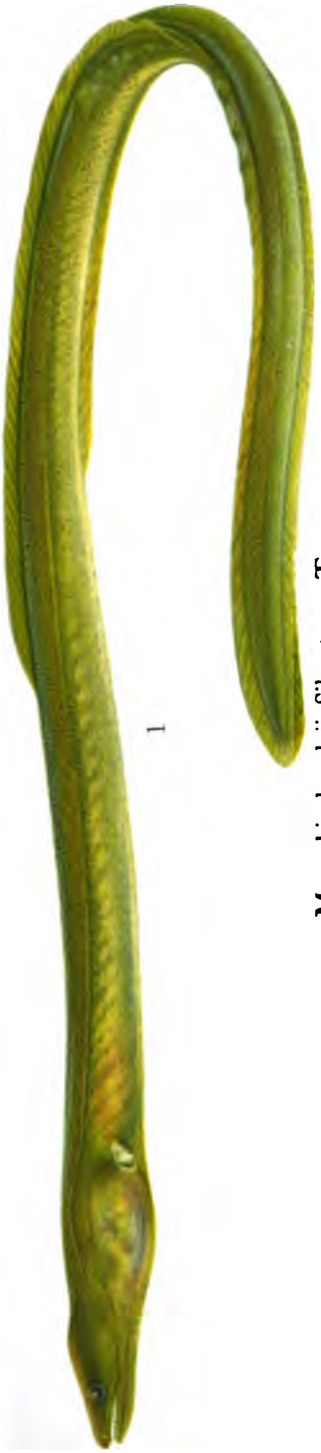
Paratype, No. 3027, Zoological Museum, Princeton University; 5 cm. long.

Named for Professor C. F. W. McClure, for his researches upon the lymphatics of fishes.



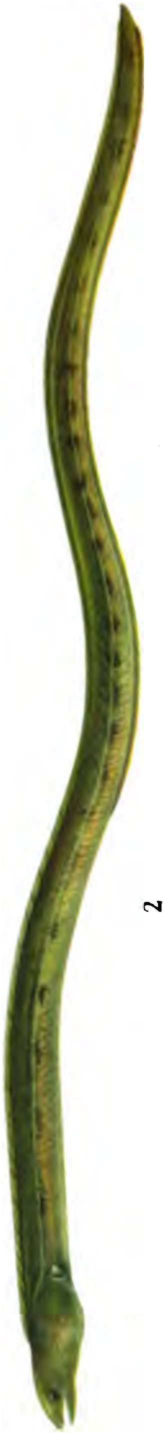
Aphthalmichthys mayeri Silvester. Type.

A. HOEN & CO. LITH



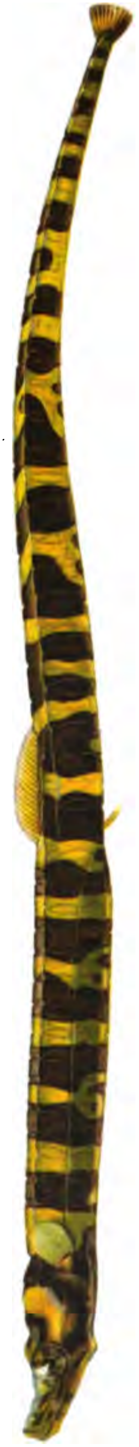
1

Myrophis longleii Silvester. Type.



2

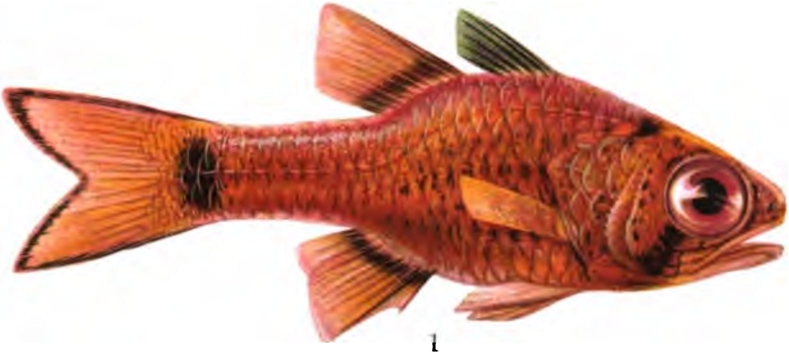
Myrichthys kecki Silvester. Type.



3

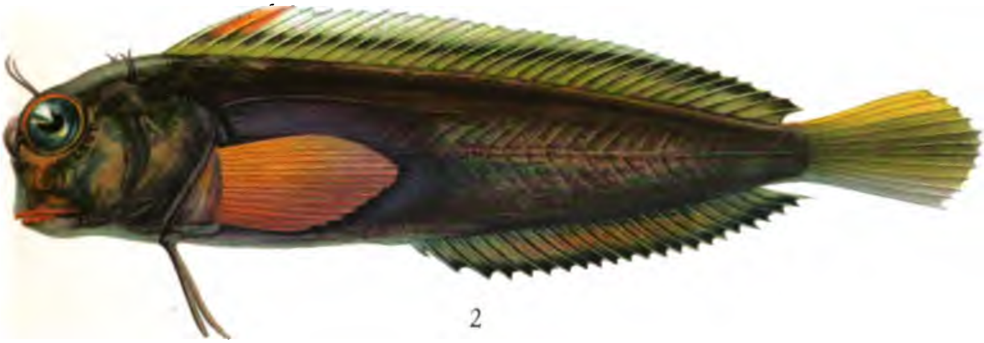
Corythroichthys ensenadae Silvester. Type.

A. HOEN & CO. LITH.



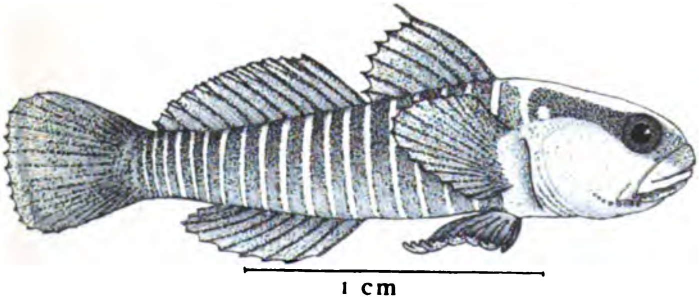
1

Amia conklini Silvester. Type.



2

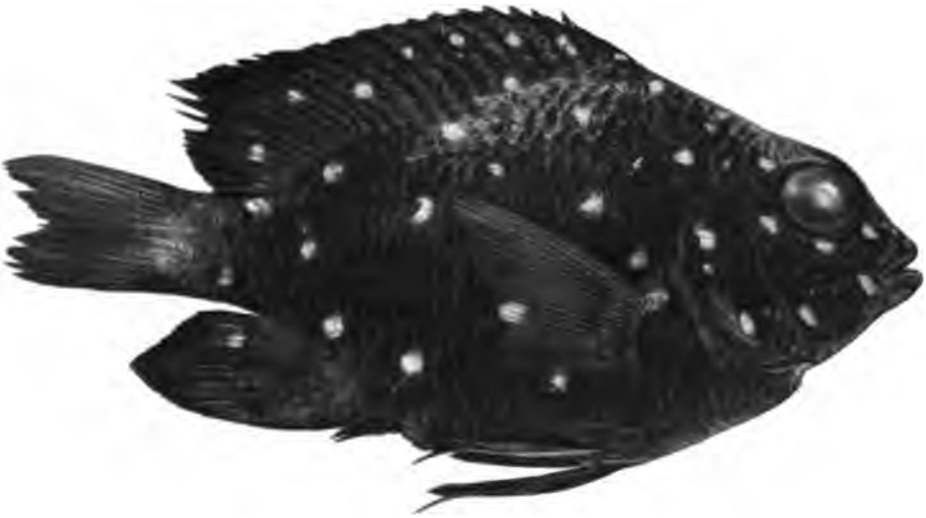
Alticus macclurei Silvester. Type.



3

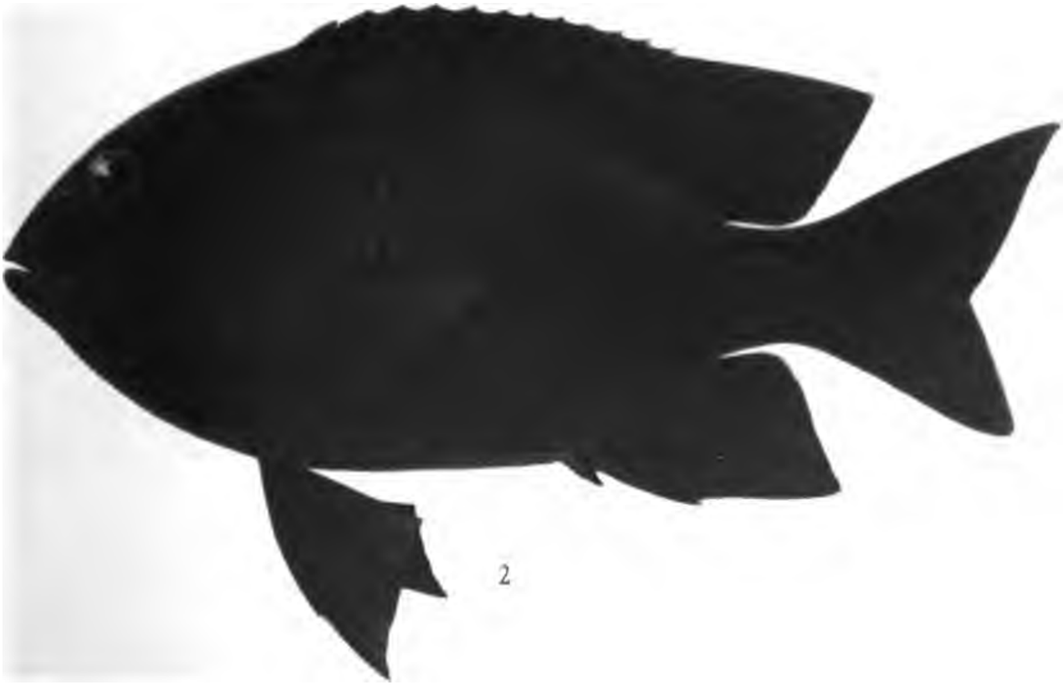
A. HOEN & CO. LITH.

Gobiosoma viridistriatum Silvester. Type.



1

Microspathodon niveatus (Poey).



2

A HOEN & CO LITH

Microspathodon fowleri Silvester. Type.

III.

ORAL GESTATION IN THE GAFF-TOPSAIL CATFISH, FELICHTHYS FELIS.

BY E. W. GUDGER,

Professor of Biology in the State Normal College, Greensboro, North Carolina.

Four plates.

CONTENTS.

PAGE.
Introduction........................ 27
Narrative........................... 27
Historical Account.................. 29
The Breeding Season................. 30
Distinction between Sexes........... 32
Method of Transfer of the Eggs...... 32
Sex of the Egg Carrier.............. 33
Size of Mouth Cavity................ 34
Size of Eggs and Number Incubated 35
Size of Young and Number Carried.. 37
PAGE.
Food and Feeding.................... 39
Feeding and Growth of Young while in the Paternal Mouth............ 42
Length of Period of Gestation....... 43
The Purpose of Buccal Incubation in the Gaff-topsail.................. 46
The Origin of the Habit of Oral Gestation......................... 46
Literature Cited.................... 51

ORAL GESTATION IN THE GAFF-TOPSAIL CATFISH, FELICHTHYS FELIS.

By E. W. Gudger.

INTRODUCTION.

In a previous paper (Gudger, 1916) I have given in some detail the story of how I came to rediscover the habit of oral gestation in the gaff-topsail catfish, *Felichthys felis* (plate I, figure 1). In order to give the facts contained in this paper their proper setting, a brief résumé of the data contained in the previous article will here be set forth.

NARRATIVE.

In the summer of 1906, while at Cape Hatteras, North Carolina, Mr. N. F. Jennett, who operated a pound-net fishery in Pamlico Sound at that place, told me that in looking over the catch of his net on the previous day he had found a number of flat-whiskered ocean catfish, and that in handling these he had found in his hands a number of young. However, he was unable to say whether these had come from the mouth or the vent. At that time I was working in the Beaufort Station of the United States Bureau of Fisheries engaged in research on the embryology and breeding habits of fishes, so on my return I at once began to look up the literature as to habits of catfishes. I found that while there was a considerable literature on oral gestation in catfishes, certain rather definite conclusions were held that the smaller ocean catfish (*Galeichthys milberti*), also found at Beaufort and well known to me, is ovo-viviparous. Further, dissection of female gaff-topsails showed that the hinder part of each ovary, and especially the duct leading therefrom, was laid in plicated folds highly vascularized, in which it seemed possible for eggs to be held and nourished until hatched. However, on talking the matter over with a number of well-informed fishermen of Beaufort, I found that it was well known that the gaff-topsail carries in its mouth its eggs and even the larvæ after hatching. One man put it that he "had seen the sea-cat spit its eggs out of its mouth." Unfortunately the breeding season was past and it was impossible to investigate these conflicting reports by study of the fish; so it was necessary to let the matter stand over until the next summer.

At the beginning of the season at Beaufort in 1907, difficulties were had in collecting early material and in finishing my observations on the breeding habits and embryology of another fish; hence it was not until June 22 that it was found possible to make a trip for catfish. In the Narrows of Newport River, at Rockfish Rock hauling-ground, a big catch of *Felichthys* was made. Thirty-two males carrying eggs were measured, and probably as many more were relieved of their oral burdens. Some half dozen of these were dissected and without exception all were found to be males. A large number of eggs was obtained, estimated at between 200 and 300, the greatest number taken from one fish being 26. These eggs were held loosely in the mouth, which was enlarged by a distension downward of the hyoid region and outward of the branchial arches, as shown in figures 2 and 3, plate I. Some of these eggs are shown in figure 8, plate III. Figure 7, plate III, is a photograph of the mouth of one of these fish with the eggs *in situ*. Attention is called to the extraordinarily distended mouth and to the great size of the eggs. One of these has two embryos.

Other trips were made during the summer of 1907, and scores of fish and hundreds of eggs were taken. Many observations and measurements of the adults were made; and much study was given to the eggs and larvæ, which were kept alive with considerable difficulty in aquaria in the laboratory. The result of all this work was the definite establishment of the fact that the male of the gaff-topsail catfish takes the recently spawned and fertilized eggs into his mouth and there nurses them until they are hatched and until the young are able to care for themselves.

During the summers of 1908, 1909, 1910, 1911 and 1912, the search for gaff-topsails was assiduously continued and no effort was spared to capture breeding males in the hope that early stages in the development of the eggs might be obtained. These efforts were attended by few successes and many failures. The failures in the seasons of 1908 and 1912 are mainly to be attributed to unprecedentedly heavy rainfall in the Beaufort region towards the close of May. These rains so freshened the water at the head of Newport estuary as to drive the catfish into the lower and broad stretches of the river, where it was found impossible to take them (despite almost daily seinings) at localities previously favored by the fish.

However, all the failures do not have so simple an explanation, for the fishermen, taking all possible precautions, made haul after haul in "holes" where recently or in previous seasons we had made fine catches, but the net came in with no catfish or at best with a few females or non-breeding males. In 1911, I even went so far as to make two trips to Beaufort; the first covering the time from May 13 to 18, the second for the season beginning May 25. The first time I was too early, the breeding not having commenced; the second time too late, the eggs having been laid during the interim.

These various failures were all the more regrettable because in the summers of 1908 and 1910, thanks to a grant from the Carnegie Institution of Washington, an artist was at hand to make drawings[1] for the embryology of the fish. However, the work went on, and gradually the series of eggs was pushed both forward and backward until now there is a complete series illustrated by photographs or drawings from invagination to the adult embryo.

---

[1] The drawings reproduced in this paper in figures 1, 2 and 3, plate I; and 9, 10, 11, 12 in plate IV, were made by Mr. E. A. Morrison under this grant.

HISTORICAL ACCOUNT.

The brief historical account herein contained will be confined to the gaff-topsail.

The first scientific man who seems to have had knowledge of this curious habit of the gaff-topsail was S. C. Clarke. In the *American Angler* for December 15, 1883, he says of the gaff-topsail in Florida: "The eggs of this species are golden yellow, and of the size of grapes, which they much resemble, in bunches of ten or twelve. The fishermen say that this catfish carries its young, when hatched, in its mouth." The following year this statement was republished in "Fishes of the East Atlantic Coast" by Van Doren and Clarke.

In 1883, and again in 1887, John A. Ryder makes brief mention of the fact that male catfish of the genus *Aelurichthys* (an old synonym for *Felichthys*) carry the large eggs (0.75 to 0.875 inch in diameter) in the hinder part of their mouth-cavities until the young are hatched. Unfortunately he gives no clue to the locality from which the eggs in his possession came.

Last of all comes Holder's (1904) brief mention of this habit in his delightful book of fishing stories entitled "The Boy Anglers." On page 175 is the following interesting statement:

> "Tom . . . secured . . . a gaff-topsail catfish . . . . As the boatman attempted to unhook it, he showed the boys its eggs packed on the inside of its mouth, where they are carried until they hatch; and even the young fish are protected in this way until they can care for themselves."

In 1908 I read a paper on this habit of this fish before the North Carolina Academy of Science, but not being ready to give out my observations, the title only was reported in the proceedings. In 1912, in the Proceedings of the American Association for the Advancement of Science for 1911, Section F, I published a short abstract giving the essential facts as to this habit, size of mouth-cavity, size, number of eggs, etc. Later I published the 1916 paper.

There is now to be given an account about which there is some doubt, but it seems best to insert it. Bloch (1794) figures (plate 365) a catfish from American waters which he calls *Silurus bagre*. This fish the Eigenmanns (1890) identify with *Felichthys felis*. In a former paper (Gudger, 1916) the question of identity has been discussed and there seems to be little reason to doubt that this identification is correct However, the point of interest just here is that on this same plate (365) Bloch has figured a little catfish sitting on a large yolk with its tail bent upward at a sharp angle, but without anywhere referring to it, though in the adjacent text he has been discussing oral gestation in *Silurus militaris*, which he figures without barbels. However, some seven years after Bloch's death, Schneider, in editing the *Systema Ichthyologiæ* (1801) of his predecessor, says in a footnote to *Silurus bagre* (p. 378): "I have observed the eggs of this fish about to be hatched in the mouth." The larval form as figured by Bloch on plate 365 has 6 barbels and lacks the filament to the dorsal fin. Whether these are errors chargeable to the artist can not, of course, be determined. Possibly this little fish is the young of some other siluroid described by Bloch in the context. At any rate it may be noted here that Bloch is the first to describe oral gestation in siluroid fishes. The whole matter is very obscure, but the data are given here that the reader may draw his own conclusions. It is interesting, however, to note that, immediately after bursting the tough eggshell, the little gaff-topsail has the same high-waving tail as that figured by Bloch. (See fig. 10, plate IV.)

THE BREEDING SEASON.

The limits for egg-laying by the gaff-topsail at Beaufort can not be set very narrowly, but an attempt will be made to indicate when males with eggs may be taken. This research was initiated the first week in August 1906, but only females long spent were taken. The real search for the fish was begun in 1907, when the first female was captured on June 4. Her ovary had in it no eggs over 10 mm. in diameter, but did show a lot of recently evacuated follicles. On June 6, other females were brought in having ova as large as 20 mm. Plainly, the breeding season was not over.

As explained before, it was found impracticable to search for the breeding males in 1907 until June 22, and the eggs taken on this day had on them young in various stages, but the majority had the whole yolk covered with the blood vascular system.

The search for early stages of the eggs began in 1908 on June 3. On this day a female with empty follicles was taken, as were others two days later. Notwithstanding daily seinings, no eggs were taken until June 11, and constant inquiries brought no news of the finding of a single egg by any of the fishermen before this date. Our failures, as explained elsewhere, were due to the freshening of Newport River by heavy rains. The youngest of these eggs had the yolk circulation covering quite half of the egg and were at least 10 days, probably 2 weeks old.

In 1909, the first eggs were taken May 27. These had on them forming embryos with a huge open blastopore behind. They were probably from 5 to 7 days old. However, a number of females were taken having enormous bellies due to the greatly swollen undischarged ovaries. A number of these females were brought to the laboratory in a live-car and kept in the turtle pound. Four days later the largest of these was spawned artificially and gave up 68 grown eggs.

In 1910, the first eggs were taken May 21, before my arrival. These were few in number and low in vitality, whether because they were naturally infertile or because they had not been fertilized could not be determined. On May 25 we were fortunate in getting males with eggs in early invagination stages. These were probably 3 to 5 days old. On the following day more eggs of about the same stage were taken.

In 1911, as noted elsewhere, two trips were made to Beaufort for gaff-topsails. Having always been too late for early stages heretofore, I went too soon this year. The first seining was done May 13 and 40 or 50 huge females were taken, but from none of them could eggs be obtained. A large number of males was also caught but not one had the depressed hyoid region indicative of the breeding season. On May 15 a number of large females were obtained but none would give up eggs. The males, however, of this day's catch had enlarged oral cavities ready to receive eggs. None of these females had genital orifices markedly reddened. This spring was a late cold one and had evidently delayed the spawning beyond the normal time.

My second trip in 1911 gave me a seining on May 25. From this were obtained the youngest eggs ever gotten in this research—eggs with the invaginating edge of the blastoderm placed equatorially. Here again I was too late for segmentation stages, the eggs having been laid in my absence. However, they could hardly have been more than 3 days old.

The breeding season is plainly determined by the stage of ripeness of the eggs and this is pretty definitely fixed by the temperature. With a warm spring, egg-laying comes earlier, with a cold one later; but the evidence seems to be that once the laying begins it is quickly concluded. Normally this breeding season, as indicated by the data above given, begins about May 18–20, and rarely extends over 10 days, probably being concluded in even less time. This is plainly evidenced by the difficulty in finding early stages, and further by the fact that the majority of eggs taken in any season at any time are all about the same stage.

There must now, however, be given some data which contrasts markedly with the foregoing. On July 21, 1910, there were brought to me, by fishermen in Newport River, 2 male catfish with eggs and larvæ in their mouths. The 5 larvæ had their yolk-sacs four-fifths closed over by the body-walls and were able to swim freely and actively.

The 28 eggs had on them embryos which were just beginning to show dark stippling on the dorsal parts, and were plainly from two to three weeks younger than the larvæ above described. This is a remarkable case. The eggs were of a late laying. With this case another must be correlated. On June 8, 1910, I took a female having ovarian eggs 7.5 to 8 mm. in diameter. These might have possibly come to maturity and would then have been extruded to give late embryos like those described above. This certainly would have been the case with the female having ovarian eggs of 20 mm. on June 6, 1907. These cases are, however, isolated ones among scores and even hundreds of normal ones, and are probably instances of extreme variation. On page 36 it is noted that the 28 eggs above referred to were markedly smaller than the normal ones.

DISTINCTION BETWEEN SEXES.

Except at the breeding season there is no way of distinguishing between the sexes by their external appearance, and so dissection must be resorted to. As the breeding season approaches the fishes begin to show secondary sexual characters. The females develop great swollen abdomens by reason of their tremendously enlarged ovaries filled with 20-millimeter eggs. However, even then there is a chance for error, since a male after a hearty meal of crabs might also present much the same appearance, though he would hardly have such an enormous abdomen as was found in the female from which were spawned 68 eggs averaging 20 mm. in diameter. As the time for oviposition approaches, the anal region of the female becomes highly vascularized, and the genital pore becomes very red and quite protuberant and is noticeably enlarged. This is a sure sign that the eggs are about ripe and ready for expulsion.

More marked, however, is the appearance of the male. Even before the eggs are received, and probably as a sexual reflex, the hyoid region of the male undergoes a marked downward deflection and outpushing (fig. 2, plate I), and the gill-covers become somewhat distended outward (fig. 3, plate I). These phenomena develop in all oral-gestating fishes. These outpushings very considerably increase the capacity of an already very large buccal cavity and are an invariable sign that such a fish is carrying eggs, is ready to receive eggs, has just cast out the eggs, or has given up the young.

METHOD OF TRANSFER OF THE EGGS.

On this interesting point nothing definite is known. In 1858 Green, and in the following year Wyman (1859), reported the finding of eggs of two species in the mouth of an oral incubating catfish of Guiana. From this they concluded that the eggs were disgorged in order to feed and later were taken up again. From this it is not a far cry to Goode's quotation from letters from Silas Stearns (Gill's revision of Goode's American Fishes, 1903): "It (*Galeichthys milberti*) breeds in summer, in June, July, and August. The spawn is deposited in a depression of the sand and impregnated with milt. One of the parent fish then takes the eggs in his mouth. . . . The eggs are carried in this position until the embryo fish are hatched." Whether this is an observation or a conjecture of Stearns's can not be said. However, the statement is very direct and positive. Smith (1907) quotes it as a fact, and it should be noted here that Stearns seems to have been a careful and accurate observer, and that he had Goode's full confidence.

However, Steindachner (1875), in describing *Arius planiceps*, a catfish from Panama, found that at the breeding season the innermost edges of the ventral fins of the female are developed so as to form a kind of pocket. He conjectured that the eggs are extruded into this, fertilized by the male, and then taken into his mouth. He found a similar structure on the female of *A. kessleri*, the male of which is also a mouth brooder. Moreover, two years before this, Day (1873), writing of the Indian genera *Arius* and *Osteogeniosus*, had described precisely similar structures and had made an identical surmise.

The present writer has sought diligently to ascertain the method of transfer in the gaff-topsail catfish, but in vain. The fish are so large that it has not been found practicable to isolate them in pairs, and the water is too muddy at their breeding-grounds in Newport River for any observations to be made in the open. No structures like those described by Steindachner have been noticed on any female examined. The manner of transfer of the eggs in this fish is still a mystery.

SEX OF THE EGG-CARRIER.

As has been stated in the preceding pages, and made clear in the article previously referred to (Gudger, 1916), it is always and only the male that carries the eggs. This has been definitely determined for the gaff-topsail by scores of dissections. In this matter *Felichthys felis* falls in line with all other siluroid buccal incubators on record save one. This one exception—probably more apparent than real—is *Arius commersonii*, a sea catfish found in the brackish waters of southern Brazil.

Hensel (1870) first made known this interesting habit in this fish and noted that the male is the incubator. Fifteen years later, von Ihering (1885) confirmed Hensel and concluded his note, which is incidental to a geographical article on the Lagoa dos Patos (in which the fish are caught), by saying that it is the female which carries the eggs. In 1888, von Ihering, in a fuller note on the fish, records the finding of eggs in the mouths of both parents, but thinks this exceptional in the case of the female, while the rule for the male. However, in 1896, he again notes that both parents incubate the eggs.

In the section of this paper on feeding, it will be shown that the female gaff-topsail is cannibalistic in tendency, being occasionally found with a stomach full of her own eggs or those of her sisters. This fact is believed to offer a solution of von Ihering's dual observations. If this be accepted as true then all known siluroid oral gestators are males.

SIZE OF MOUTH CAVITY.

While this, of course, varies with the size of the fish, it is always large, since the head of this fish is much larger in proportion to the size of the body than is generally the case in teleosts. This may be easily seen by turning to plate I, figure 1; to the dorsal view of the head, plate I, figure 3; and to the figure showing the mouth with embryos, plate III, figure 7.

Incubating males vary greatly in extremes of size. The smallest ever taken by the writer was 13 inches long over all and carried 4 eggs; while the largest was 23 inches in extreme length but carried no eggs. However, the condition of his hyoid region indicated that he had just given up or was about to receive eggs. The largest number of eggs taken from one male was 55, the fish being 22 inches long. However, the average size of adult egg-carriers is remarkably uniform, running from 18 to 21 inches. On the other hand adult breeding females run larger, from about 19 to 24 inches.

Casts were made of the buccal cavities of 5 nursing males, and 4 of these will now be described seriatim. The first, a cast of plaster of paris, with a volume of 140 c.c., was made from an 18-inch male. The second, also of plaster, is of the mouth of a male 18.75 inches long carrying 11 eggs. Its volume is 135 c.c. The third, of the same material as the preceding, was at the time thought to be the maximum of size since it had a displacement of 267 c.c. Unfortunately the notes giving the size of the fish and the number of eggs carried have been lost. However, these may easily be omitted since full data can be given for the fourth fish, the one from whose mouth 55 eggs were taken.

This fish (22 inches long) was brought to the laboratory that a plaster cast of its enormous "Keimhöle" might be made, but there was not enough plaster in the laboratory to fill it, and there was none in Beaufort. In this predicament the director of the laboratory, Mr. Henry D. Aller, came to the rescue with the suggestion that a cast be made of Portland cement, a barrel of which was at hand. This was done and the fish was held with a towel wrapped around its gills to prevent the escape of the semi-liquid cement until it had hardened. The head was then cut off and put in a dense cedar thicket under a box where it remained until the ants had eaten off all the flesh. Then the cast was carefully freed of the disarticulated bones, shellacked and preserved.

This cast, shown in dorsal, lateral, and ventral views in figures 4, 5, and 6, plate II, is enormous, exceeding in volume both the next largest taken together. Immersed in water up to the deep insinking made by the sphincter muscles of the œsophagus, its displacement is 580 c.c. The volume of the average-sized egg is 3.75 c.c. Fifty-five eggs would have a total volume of 206.3 c.c. Allowing one-third of this additional for the interstices between the eggs, the total space occupied by the eggs was 275 c.c., leaving 315 c.c. not occupied.

The mouth-cavities of the fish carrying these eggs were very large to begin with, since, as has been noted, the head makes up a large part of this catfish, and these cavities were still further enlarged to accommodate the considerable numbers of immense eggs carried therein. Careful dissections have brought to light the following points: (1) the gill-covers, while outwardly showing no very marked distention, inwardly stand out and away from the gills by about half the diameter of an egg; (2) the gill-arches are pushed outward, giving them a sharp boomerang-like bend in the center; (3) the hyoid cartilages are greatly depressed, forming an outpushing which has been elsewhere referred to as a "double-chin" as shown in the drawing of this fish, figure 2, plate I; (4), that part of the buccal cavity, behind the pharyngeals and back of the last gill-arch, extending to the point where the sphincter muscle shuts off the œsophagus, is of considerable volume, and in it are always to be found the last eggs, which are always more or less hard to extract. This was particularly noted in the season of 1909. The males could be held up by the tail and shaken without setting all of the eggs free. In a large fish there is space enough back of the last gill-arch for a number of eggs to be held snugly by the mucous lining of the pharynx.

The above measurements, while accurate enough for the casts, can only approximately give us the sizes of the buccal cavities of these fish. That some error is to be discounted is clear from the process of making the casts as given on page 34. The plaster casts are probably too small, owing to too great constriction in the opercular region; while the cement cast is probably somewhat too large, owing to the distention caused by the large amount of heavy cement.

SIZE OF EGGS AND THE NUMBER INCUBATED.

The eggs, while enormously large, show considerable variation in size, running from 15 to 25 mm. in diameter. Heretofore, however, sufficient measurements have not been made to enable one to say with any definiteness what is the size of the average egg of *any* oral gestator. For the gaff-topsail hundreds of measurements have been made and from them the following diameters are given as covering both normal and abnormal sizes of eggs. It has been constantly observed that the non-fertile eggs of this fish are much larger than the fertile ones. In 1908, at one catch 11 such eggs were taken and measured in 3 diameters. They ran in millimeters, 17 by 18 by 20, 18 by 19 by 20, 18 by 19 by 21, 18 by 20 by 21, 19 by 20 by 21, 21 by 21 by 22, 22 by 22 by 22, 22 by 22 by 23 (two), 22 by 22 by 25, 23 by

24 by 27. The largest fertile eggs measured 18 by 18 by 19, 19 by 19 by 20, 19 by 19 by 22, 20 by 20 by 21. Incidentally these measurements show that the eggs are rarely round, some are markedly elliptical—one 22 by 22 by 26 mm.

To ascertain the average size, the following data are selected out of the large amount available. Of live eggs 138 were measured as follows: Longest diameter only: 17.5 mm., two eggs; 18, nine; 18.5, eleven; 19, thirty-nine; 19.5, ten; 20, thirty-eight; 20.5, eight; 21, nineteen; 21.5, twenty-two. Average of 138 live eggs 18.8 mm. Of preserved eggs, 189 were measured as above: 16.5 mm., three eggs; 17, one; 17.5, one; 18, four; 18.5, two; 19, thirty-four; 19.5, eleven; 20, sixty-four; 20.5, ten; 21, forty-nine; 21.5, five; 22, five. Average of 189 dead eggs, 20 mm. The average of the 327 eggs is 19.5 mm. From the above it will be seen that the largest number of eggs measure 19, 20, and 21 mm. in longest diameter, the 20-mm. eggs being the most abundant—102 out of 327. Normal eggs of this size are shown in figure 8, plate III, while the egg of figure 9, plate IV, is about the same size but has an older embryo.

In connection with the foregoing data, the following measurements should be given, since they are of the smallest eggs, taken as a lot, which have been found in the six years' search. They measured in longest diameter: 15 mm., one; 16, three; 16.5, one; 17, four; 17.5, one; 18, ten; 18.5, one; 19, seven. The average for the 28 eggs is 17.7 mm. This average, however, would be much lower if the last three lots of eggs of normal size were omitted, or even the one last lot only. These eggs were taken on July 21, 1910, and contained embryos having black stippling on the dorsal surface. At the same time there were obtained, from another fish, 5 larvæ which seemed to be from 2 to 3 weeks older than these embryos. All these things lead to the conjecture that possibly these smaller eggs are a lot coming to maturity later and are of a second laying. On these points see page 31.

Not only is there great variation in the size of the eggs, but also in the number carried. Twice I have taken fish with only 2 eggs in the mouth. The smallest incubators caught during this research were 13 and 15.5 inches over all. Each carried 4 eggs. Other small numbers of eggs incubated were 2, 5, 8, 11 (in 2 fish), 13, 14, 15 (in 2 fish), 16, etc. The largest number found was 55 in a 22-inch male. Notable also are 50 (22-inch fish), 45 (taken twice), 38 (22- and 20-inch fish), 36 (from a notably small fish 17.25 inches long), 35 (22-inch), 32 (fish, 20.25 inches long), and numerous catches in the twenties. Once a large male was found with only one egg in his mouth (the only instance anong hundreds captured), but from the great size of the buccal cavity it is probable that other eggs had been thrown out.

That these large numbers of colossal eggs do not, however, entirely fill the capacious buccal cavities of these fish may be readily perceived by referring to the calculations given on pages 34 and 35.

Before leaving this subject brief reference may be made to the large size and to the number of eggs which are carried by other siluroids practicing oral gestation. Evermann and Goldsborough (1902) noted that 39 eggs were taken from the mouth of a 13.5-inch male *Conorhynchus nelsoni*. The average size of the eggs measured was ten-sixteenths by eleven-sixteenths of an inch. Von Ihering (1888) found eggs of 18 mm. diameter to the number of 3 or 4 dozen in the mouths of "large" males of *Arius commersonii* (*Netuma* or *Tachysurus barbus*), a salt-water catfish of southern Brazil. Day (1873) obtained 15 to 20 eggs, 0.5 to 0.6 inch in diameter from the mouths of *Osteogeniosus militaris* and various species of the genus *Arius* in India. Boake (1866) reported the eggs of his Cingalese *Arius* to be about the size of small grapes. Günther (1864) says that the eggs of *Arius fissus* of Cayenne are rather larger than peas. Last of all, but not least, are the eggs of that catfish of Guiana whose native name is *jarra-bakka*, which are declared by Wyman to measure 0.75 inch in diameter. However, it may be remarked that, although much work has been done in recent years on oral gestation in the catfishes of Guiana, none with eggs so large has ever been taken.

SIZE OF YOUNG AND NUMBER CARRIED.

It is to be regretted that no such full data can be given for larvæ as for eggs carried by the gaff-topsail paterfamilias. Unfortunately but few lots of larvæ were taken, and these larvæ were rarely killed at the time of capture, but were kept that their behavior might be studied, their organogeny worked out, and drawings of them made.

On July 18, 1907, an 18-inch male was caught and from his mouth were taken 21 larvæ. One of these, of average size, after being in 10 per cent formalin for 24 hours, measured: extreme length (point of snout to tip of upper lobe of caudal) 57 mm.; outside width over eyes 11.25 mm.; length of dorsal spine 14.75 mm.; yolk-slit, longitudinal measurement 18 mm., transverse 16.5 mm.

On July 7, 1908, an ovigerous male was taken in whose mouth were found 6 larvæ measuring 53 to 55 mm. long over all. On July 21, 1910, 5 larvæ were taken from the mouth of a male, but unfortunately neither father nor young was measured. Young of about this stage are shown in figure 11, plate IV.

As to the size the young attain before leaving the shelter of the paternal mouth, fairly definite data can be given. In general it may be stated that departure does not occur until the fishlet is grown, *i. e.*, until the yolk-sac has been inclosed by the body-walls. This *a priori* conclusion was verified when, on July 20, 1908, my fishermen brought in a young catfish which they had taken from the mouth of an adult some hours before. In this fish, which was about 4 inches long, the body-walls had completely closed over the yolk of which there was no outward sign save for a fine aldermanic curve in the abdominal region. The escape overboard of the little fish in the transfer to my bucket forestalled the taking of any measurements. The fishermen (men in whom I have large confidence) told me that towards the close of a haul they had noticed one big catfish and a number of little ones swimming around in the net. The little ones got away but the big one was hastily secured and in his mouth was found the 4-inch young one referred to. A little catfish in this stage of development is portrayed in figure 12, plate IV.

In this connection the following incident may be related, the narrator being long and favorably known to me. Mr. T. E. Adair says that in the early nineties, he and his brother Charles were seining one day off the mouth of Wading Creek in Newport River, when their seine brought in a number of gaff-topsails. These spat out a lot of young some 2.5 or 3 inches long "with a great big yolk dividing the little fish in two." Then he and his brother both distinctly saw the old one open his mouth and the little ones swim into it. He then "took up the big one and poured out a double handful of young ones."

In the section on size of mouth cavity, it was stated that 5 casts had been made but only 4 were described; the fifth cast was that of the mouth of the 18-inch male brooding 21 larvæ as previously noted (p. 37). The volumetric displacement of one of these larvæ, for which measurements have been made, was 4 c.c. That of a slightly smaller one was 3 c.c. scant. Thus the total volume of the 21, if they averaged 4 c.c., would be 84 c.c., or if 5 c.c., only 105 c.c., while the capacity of the mouth as shown by the plaster cast was 120 c.c.

In intimate relation with the number of eggs and young carried by an adult male is the question of how many eggs an adult female may extrude. The largest number of eggs gotten is 55 from the mouth of a 22-inch male. Only one adult female has ever been spawned. This fish is merely noted in my records as being very large (probably about the size of the male above) and from her were obtained 68 eggs. Whatever the facts for smaller fish, it would seem that the largest male does not carry in his great buccal cavity as many eggs as the largest adult female can produce. From which it seems probable that the fish are polyandrous as well as polygamous.

The data at hand indicate that not all the ova extruded are fertilized, and it is quite probable that all the eggs fertilized are not hatched, and that all the young hatched are not matured. However, it would seem from my experiments that, when once the eggs are fertilized, the mortality in the paternal mouth is far less than in the best-regulated hatching-jars. Considerable data on this point have been previously given (Gudger, 1916).

FOOD AND FEEDING.

From the peculiar dental armature of the gaff-topsail (the teeth being confined to villiform bands on the vomer and palatines and to supra-pharyngeal and infra-pharyngeal pads, and hence adapted for crushing rather than biting, tearing, or holding), one would conjecture that it is not a feeder on fishes, a predatory fish in the common acceptance of the term, but rather a bottom feeder. This finds confirmation in the fact that it prefers a muddy or sandy bottom in muddy water where it finds its food by the help of its tactile organs, the barbels. Moreover, dissections of scores of both males and females make it clear that the gaff-topsail feeds almost exclusively on crustacea. Autopsy reveals an occasional fish (menhaden or croaker), but its principal food is crab, blue crab, eked out with an occasional shrimp. From the much-distended stomachs of sundry specimens, crabs 4 to 5 inches wide have been excised, so large that one wonders how they could have been swallowed. Ocular examination of the contents of the intestines has shown large fragments of the chitinous coverings of crustacea with here and there a claw. On the whole, these contents present a felt-like appearance which persists under an ordinary eye-glass. The microscope, however, resolves this material into minute fragments of chitinous shell, grains of sand, bits of dirt so small that they give the Brownian movement, and immense numbers of crustacean hair-like setæ. There can be no doubt that crustacea large and small form the major portion—perhaps as much as nine-tenths—of the food of the gaff-topsail catfish.

The foregoing observations have been made on females and non-breeding males. Such have always been found in fine full-fed condition, the intestinal tract being plump, well-nourished, fat, distended. Breeding females do not have the distended stomachs of the non-breeding ones, the size of their colossal ovaries forbidding this, but all have been found in good condition.

Breeding males, whether carrying eggs or not, may always be recognized at a glance by their depressed hyoid regions, their "double-chins." For those without eggs it may be conjectured that they have through fright or some mishap given up their eggs, or that they have not yet received them but are prepared therefor—in similar fashion as the uterus of the stingray (*Dasyatis say*) becomes villous to receive the egg even before the latter descends into the oviduct. Such breeding males always have empty, pendulous stomachs and stringy intestines without trace of food in them. This has been found the case in more than a hundred autopsies. From these facts the conclusion is drawn that the ovigerous males of *Felichthys felis* do not feed at all during the time of gestation. Certain it is that no body of any size can pass down the œsophagus without the eggs following.

Holder (1904), however, speaks of the fish with eggs in their mouths biting ravenously at hooks baited with shrimp. But Evermann and Goldsborough (1902) expressed the opinion that *Conorhynchus nelsoni* does not feed during gestation. Day (1873) found no trace of food in the intestinal tracts of either *Arius* or *Osteogeniosus*, both oral gestators of India. Boake (1866) concludes that the Ceylonese *Arius* may feed on microscopic "nutritious particles" floating in the water, but Turner (1867), after studying Boake's specimens, thinks that the amount of food obtained in this way would be practically nil. On the other hand, Wyman, though inclined to think that gestating males fasted, from finding in the mouth eggs of another species was led to believe that the fish might have disgorged the eggs in order to feed, and that on taking them up again the eggs of "another species" were also included. The wording of his sentence indicates that the other species was also a catfish. If this be true it may be conjectured that these eggs were the smaller unripe eggs of the female from whose ovary came the other ripe eggs, extruded and taken into the mouth of the male at the same time with the ripe eggs.

Save Holder (1904), previously referred to, the only other investigator, in all the literature of oral gestation known to me, who has found an incubating male to feed, is von Ihering. He (1888) speaks of catching females of *Arius commersonii* on the hook, but says that males whose mouths are filled with eggs do not take the bait except on rare occasions. Excepting these authors, all writers agree that fishes practicing buccal incubation fast during this period. Such is my conclusion arrived at after 6 years' study and research on the gaff-topsail. In this fish the incubatory period has not been definitely worked out since it has been found impossible to carry early eggs through hatching to the stage of independent life. However, so far as it has been made out by piecing together my observations, incubation continues about 70 days.

Many authors have noted that the incubating fish at or toward the close of gestation are thin, emaciated, worn out with their labors. Unfortunately I have taken but few gaff-topsails in late incubation, and none towards its close, and have noted no striking emaciation in the fishes captured. A few, however, have been rather thin and in poor condition, and moreover it has always been noticed that incubating males taken in June and July do not seem to have much stamina. After being relieved of their eggs they die very quickly, whereas females and non-breeding males are very active and robust, and as a fisherman once remarked "require a good deal of killing" with a fish club before they become quiet. From this it would seem that the duty of incubation bears rather heavily on the male.

The foregoing observations and conclusions have been based on normal fish in normal condition, but before leaving this subject it is necessary to relate and account for certain abnormal cases coming under my observation. These are not only interesting *per se*, but offer a probable explanation for an error of nearly 100 years' standing.

On July 25, 1907, I found in the stomach of an ovigerous male a perfect egg and a fragment of another. Neither showed the faintest trace of the action of the gastric juice. On July 15, 1908, a male was taken and, after removal of the eggs from the mouth, autopsy showed the presence in the stomach of a perfect egg plus several fragments, none of which showed any trace of digestion. In 1909, dissection revealed in the stomach of a male fish a whole perfect egg having an embryo in the same stage of development as those taken from the mouth. This egg was so sound and normal that I expected to see the little fish wriggle on its yolk. Other than this the stomach contained nothing but a whitish ropy mucus. The small intestine was very much reduced in size, inconspicuous in appearance, and was empty but for some yellowish-green watery material. The belly was lanky, but there was no other evidence of wasting or emaciation. In 1910 another male was taken having 2 crushed eggs in his mouth and the fragments of 3 or 4 more in his stomach. Here again there was not the slightest evidence of digestion.

To the writer three possible explanations of the above phenomena offer themselves. (1) In the excitement engendered in the endeavor to escape as the bunt of the seine comes in filled with floundering fish, the male gaff-topsail may give off part of his eggs and swallow others. (2) When the fisherman grasps the fish by the mouth to prevent the loss of eggs, the fish may swallow some in its writhings. (3) Most of the fish just described had been struck on the head with a fish club to quiet them. If there were any eggs between the pharyngeals at this time they would be crushed; further, the blow by reflex action might cause the sphincter muscles of the œsophagus to relax, whereupon both eggs and fragments might be swallowed.

In none of these cases is there any evidence of the eggs having been swallowed as food. Herein my observations and conclusions agree with the only others on record in this matter. Wyman (1859) writes:

> "Besides some nearly mature fœtuses [of *jarra-bakka*] contained in the mouth, two or three were squeezed apparently from the stomach, but not bearing any marks of violence or of the action of the gastric fluid. It is probable that these found their way into that cavity after death, in consequence of the relaxation of the sphincter which separates the cavities of the mouth and stomach."

There are now to be described, and if possible explained, three extraordinary cases which do not come within the above category.

On May 29, 1911, a large fish with a great belly was captured. Touch showed the presence of eggs in this, but they seemed too far forward to be in the ovary. Autopsy made the matter clear. The fish was a male whose stomach contained a great mass of eggs stuck together like a bunch of grapes. These eggs, measuring 18 mm. in diameter, were perfectly fresh and with them was a large number of small eggs and empty follicles, such as are extruded when a female is spawned by pressure applied to the abdomen. Evidently they had been taken up by the male immediately after extrusion by the female and had been swallowed presumably as food. The eggs at this stage are apt to be adhesive and to clump in masses. This was found to be true in the case of the great female spawned artificially, as has been noted elsewhere.

The other two cases may be considered together. A giant fish with an enormous belly was taken on May 25, 1910. Eggs could be plainly felt, but could not be spawned. Dissection revealed that the fish was a female and from her stomach were taken 23 whole and 6 half eggs together with small eggs and partly digested fragments equal in bulk to all the others. On the following day another large fish was taken which also could not be spawned. On performing an autopsy her stomach was also found crowded with 23 full-grown and a large number of small eggs, none of which, however, showed traces of digestion. In both cases it seems probable that the eggs had been swallowed immediately after extrusion and as food; hence we may conclude that the females, unlike the brooding males, are cannibalistic.

These fortunate catches seem to the writer to offer the explanation of the allegation by Hillhouse (1825) and others, especially Bleeker (1858), that certain catfishes are viviparous, the evidence being that they have seen eggs and fœtuses cut out of the bellies of catfishes of various species and genera. These are probably cases in which the eggs and fœtuses had been swallowed by the fish as food.

Possibly the most unique find of contents of stomach was made June 16, 1908. A male taken on this day was found by touch to have some hard object in the stomach. Autopsy revealed a big wooden splinter or fragment some 5.25 inches long. The stomach was empty except for this and a considerable amount of mucus. No suggestion can be offered concerning the presence of this anomalous body in the stomach of this fish.

FEEDING AND GROWTH OF YOUNG WHILE IN PATERNAL MOUTH.

At the time of hatching, *i. e.*, bursting of the shell, the young measure about 45 mm. over all, but when they leave the mouth and begin an independent existence they are about 85 to 100 mm. long. An early egg, 20 mm. in diameter, after having been in 4 per cent formalin for 2 years and 6 months, weighed 3.5 grams. A larva with body-walls which had just completely closed over the yolk, the seam of closure only being visible, after being in 4 per cent formalin for 4 years and 3 months, measured 93 mm. and weighed 9 grams. This fishlet was in all respects normal and was just in the stage when the young begin to lead a free life. To make this point clear, the reader is asked to contrast the young fish in figures 11 and 12 of plate IV with the eggs with comparatively early embryos shown in figures 8, plate III, and 9, plate IV.

This increase in length of over 45 mm. (a doubling) and in weight of 5.5 grams (almost a trebling) can not be accounted for by yolk alone—*the young feed in the mouth of the parent.* At some future time it will be shown that the larvæ reared in running sea-water grew faster and when fed by hand were less voracious than those kept in a diluted sea-water filtered many times, the latter feeding ravenously when given bits of oyster. The conclusion drawn in 1907, which has remained unchanged, is that the young feed while in the mouth of the father by filtering out of the respired sea-water, by means of their closely set gill-rakers, minute crustacea to satisfy their hunger. In this way only can their great increase in size and weight be accounted for.

Von Ihering (1888) is the only investigator who has given any data whatever on the growth of the young while in process of brooding. Of *Arius commersonii* he records that the eggs in early development stages average 2.5 grams, while at a time when the larvæ are 60 mm. long they weigh 4.3 grams. The increase in weight, he argues, is due to the intake of nourishment by the embryo.

LENGTH OF THE PERIOD OF GESTATION.

It is impossible to state this either from observation or direct experiment. It being impracticable, at the time this research was carried on at the Beaufort Laboratory, to isolate the fish in pairs, direct observation of the period of incubation was not feasible. All efforts to effect artificial fertilization proved abortive, nor was I ever able to get eggs in early segmentation stages. Thus it is impossible to fix the time of fertilization, which would give the time of the beginning of incubation. Further, it has been impossible to carry any one set of early eggs through to time of hatching (*i. e.*, bursting the egg-shell) and of the complete closing of the body-walls over the diminished yolk-sac as shown in figure 12, plate IV, at or about which time the young are set free from the paternal mouth.

Confronted by these impossibilities, the best that can be done is to make the closest approximation possible from the data at hand. Now it has been shown in the section on the breeding season that the eggs are "laid" under ordinary conditions of weather (*i. e.*, temperature) between May 18 and 30, and more narrowly between May 20 and 25. There are exceptions, but on the whole the breeding season is seemingly concentrated within narrow limits and the period of incubation has its approximate beginning within these 10 days.

The information at hand is even less definite as to the time of cessation of the care of the male parent. The mortality of eggs hatched in aquaria is very great, and with young eggs all may be expected to die. In MacDonald hatching-jars, which have a bowl-shaped base with water admitted at the center of the base, the eggs are lifted by the incoming current, and all sediment, mold spores, and bacteria are carried off by the overflow at the top. It was thought, moreover, that the use of these jars would conserve the life of the eggs in another way. When the yolk-circulation has extended its plexus of blood vessels over the ventral side of the eggs, if these are allowed to stand in one position, shortly a congestion of these ventral blood vessels is noticed and this is quickly followed by the death of the eggs. In the mouth of the father the constant current of water due to the respiratory actions keeps the eggs free from sediment, bacteria, etc., and presumably keeps them in motion, thus preventing the congestion referred to. It was hoped that in the MacDonald jars the uplifting action of the water would keep the eggs "dancing" and prevent this trouble. To some extent this hope was realized. But even in the best-handled jars, although there was little congestion, the eggs turned white and died by dozens and scores, and in no case was it ever found possible to carry any but late eggs on to hatching. Eggs brought in with young nearly ready to burst the shell could with great care be carried through this dangerous period, and if carried 3 or 4 days further, little trouble was had in bringing them on to maturity. It is interesting to note, however, that there was never any evidence of death due to bacteria, as has been found in the eggs of the toadfish, *Batrachus tau*. For the details of the difficulties met with in the effort to hatch eggs, the reader is referred to the author's 1916 paper.

However, although no definite time can be set for the liberation of the young from the mouth of the father, this can at least be approximated as the time when the body-walls have closed up, as shown in figure 12, plate IV, and the young fish set out to find their own living. In 1907, on July 29, a little catfish was killed and hardened in formalin for 15 hours, at the end of which it measured 81 mm. long over all, while the yolk-slit measured 16.5 mm. long by 2 mm. wide. On August 1, when I left Beaufort, the other fish of this lot in a large aquarium were relatively as active as the adults in Newport River: their babyhood was over.

In 1908 two sets of larvæ were under observation at the close of July. The older ones had the yolk covered over and the slit reduced to a mere seam on July 27; the younger ones, however, on the same day had the yolk only half covered. On August 8, the day before my departure, the body-walls of the younger had nearly closed over the yolk, while the older had bellies showing no more protuberance than is to be found in the adult shown in plate I, figure 1. These younger eggs were plainly of a late laying.

In 1909 I left the laboratory on July 3 and hence have no data for hatching this year other than that at this time the little catfishes were just beginning to burst their confining shells. However, the fuller data for 1910 show that on July 21 two lots of eggs were taken from a male; one with young embryos just beginning to show dark stippling, while the other lot had embryos with the body-walls covering about half of the yolk; these were from 2 to 3 weeks older than the former. See further on this point page 31, in section on the time of breeding. On July 30 an older fish measured 3.75 inches over all and had the edges of the body-wall united to make a raphe on the mid-ventral wall. The more advanced of the younger lot were able to skate on their yolk-sacs (see figure 11, plate IV), while others were not able to move these yolks which still anchored them to the floor of the aquarium.

All the evidence at hand indicates that the mortality in the ancestral mouth is far less than in even MacDonald hatching jars, and that the young brooded therein become free-swimming earlier. This is undoubtedly due to the fact that they feed while therein as previously described. Only once have I had a fully fledged young one from the mouth of the father. On July 20, 1908, my fishermen, men whom I know intimately and in whom I have great confidence, brought me a catfish about 4 inches long which they had gotten from the mouth of a male. It showed a mere seam on the flattened belly, the yolk being completely gone. No exact measurements could be made, since during the examination the fish jumped overboard and swam away like a flash.

From the data presented above it would seem that the time for the liberation of the young is from July 20 to 30, and under ordinary circumstances about the last week in July. Recalling that incubation begins May 20 to 30, we can approximate the length of the period of gestation as from 60 to 70 days. However, it is clear that the matter of the maximum or minimum limit will be largely if not wholly determined by the temperature of the water and the amount of small crustacea and other microscopic organisms contained therein and available as food for the larvæ.

THE PURPOSE OF BUCCAL INCUBATION IN THE GAFF-TOPSAIL.

This may be summed up in one word—protection. Let it be recalled that these eggs are of enormous size (the average diameter of 327 eggs being 19.5 mm.) and that when in middle embryonic stages they are very attractive to the eye because of their blood-red vascular yolk investment. For these reasons, if laid as other fish eggs are, they could hardly be expected to escape the eyes of marauding fishes, but if any were so fortunate they would almost certainly be eaten by crabs, those scavengers from which practically nothing escapes. The result would be the inevitable extinction of the species. Moreover, there is yet another danger to which the ova are exposed. These catfish spawn and spend the hatching season on mud flats. If the eggs were discharged on such bottoms they would (because of their great weight, averaging 3.5 grams) sink into the mud and be smothered. To avoid these various dangers, these fish have to do one of two things to insure their perpetuity, *i. e.*, to practice mouth gestation or to lay their eggs in nests which are guarded by one or both the parents. Some fresh-water catfishes have adopted the latter habit; the gaff-topsail has chosen the former.

The whole matter, barring his ignorance of the habit of buccal incubation, has been admirably put by Gurley (1902).

> "It is almost impossible that a mud bottom should be a successful spawning-ground, as the eggs will almost inevitably be asphyxiated. Wherefore, fishes experiencing an impulse to spawn on such bottom will leave few descendants to inherit their delicately sensitive mucous membrane, while those having an impulse to seek harder bottom will transmit to a larger progeny their more roborant mucous membrane. Further, the exception sustains the rule, the only species spawning on mud bottoms being certain catfishes, the males of which excavate nests, and attend to (probably aerate) the eggs, and care for the fry."

THE ORIGIN OF THE HABIT OF ORAL GESTATION.

The causes leading to the practice of buccal incubation are plainly set forth in the preceding section; the eggs must be guarded in a nest or carried in the mouth, or else the fish would soon become extinct. The gaff-topsail has chosen the latter means to maintain itself. But how has the habit been developed? The answer to this is largely conjectural, but there is a fair amount of data to be adduced upon which to base our conjecture.

It has long been known that some fishes pick up their eggs or young in their mouths for transport from place to place much as a cat does her kittens or a fox her cubs. The writer believes that in this habit are to be found the beginnings of oral gestation. The fishes referred to are members of the families Gasterosteidæ and Osphromenidæ, together with one or two other isolated forms.

The first account to be given is the classic one of Coste, "Nidification des Épinoches et des Épinochettes," published in 1848. After describing the making of the nest and the laying of the eggs, Coste tells how the male *Gasterosteus pungitius* stands watch and ward over the nest until the eggs are hatched, hastening the process by fanning them free of sediment and by helping in their oxygenation at the same time. When hatched, "He does not allow the young to go outside the boundaries of the nest, and if any one does so, he takes it in his mouth and bears it immediately back to its domicile. If, however, the number of deserters increases, he seizes several at one time without hurting them." With regard to the question of feeding during this self-imposed guardianship, Coste adds:

> "This animal, which during all the remainder of the year is remarkable for its voracity, suffers an abstinence almost complete during the time devoted to the construction of its nest, the care of its eggs, and the training of its young."

An almost equally charming account is that of Albany Hancock (1854) for *Gasterosteus aculeatus* and *G. spinachia.* In most interesting fashion he describes how he saw the watchful parent with gaping jaws seize the little wanderer, who disappeared therein, as he thought, forever. But to his delight the old fish returned to the nest and deposited the small straggler therein. Then he saw that it was the purpose of the paterfamilias to allow no rambling from the nest. Sometimes the fry were held in the mouth for an appreciable length of time, but they were never harmed.

A similar state of affairs was noted for *Gasterosteus leiurus* by Robert Warrington in 1855. After describing the building of the nest, he says that as the yolk-sacs of the developing young become smaller and their activity greater, "their attempts carried them to a great distance from the parent fish; his vigilance, however, seemed everywhere, and if they rose by the action of their fins above a certain height from the shingle bottom, or flitted beyond a certain distance from the nest, they were immediately seized in his mouth, brought back and gently puffed or jetted into their place again."

Furthermore, if the fishlets are removed by artificial means the father brings them back in his mouth and shoots them into the nest, according to the observations of William Houghton, recorded in 1865. Ransom, in the same year noted the same habit and says of *G. pungitius:* "He seemed to take no food." "The Tinker was almost starving in the midst of plenty." Nor are later observations of this habit lacking if one may credit Becker's notes on *G. aculeatus* published in 1907.

Almost in the Antipodes, we find certain osphromenid fishes practicing similar habits. Many of these make floating nests of foam and mucus in which the male guards the eggs and young. The first account is from the pen of the indefatigable French aquarist of the third quarter of the last century, P. Carbonnier. Describing a Chinese macropode (1869, 1869*a*), he writes of the male that, after extrusion and fertilization of the eggs:

> "He patiently gathers in his mouth the eggs scattered on all sides and carries them under the roof of foam which becomes then for some ten days the object of his solicitude. Without even taking food, he passes his time in watching over the receptacle of his progeny. If one part of the nest begins to empty itself he fills it with new bubbles; he withdraws the eggs where they seem to him to be in too great numbers and carries them into empty places; with blows of his head he disperses the eggs if too much accumulated. When hatching is over he watches with some care over the young embryons; he chases down those which leave the protecting roof, and holding them in his mouth brings them back. He does not cease this surveillance until the too large number of fugitives announces to him that his part is played and that the young family is able to look after its own protection."

Again, in 1872 Carbonnier described similar habits for a "Macropode of China," which may or may not have been identical with the fish described above. In this case, however, both the male and the female took up the young. "I saw the female place in the mouth of the male the slender little [sick] fry which would have certainly perished without intelligent paternal care." Some years later Carbonnier (1875, 1876) describes how the paternal rainbow fish, *Colisa arc-en-ciel,* collects the scattered eggs and embryos in his mouth and transports them to the nest.

Belonging to the same group of fishes is the fighting fish, *Betta pugnax,* whose breeding habits are interestingly described by Waite (1904). This fish is so named because it is bred and trained for fighting by the Siamese. Waite describes in detail its breeding habits, but we are interested here only in its collecting the scattered eggs in its mouth and transporting them to the nest. Waite also (1905) finds a similar habit practiced by the paradise fish, *Polyacanthus opercularis,* the other Macropodes being probably cultivated varieties of this fish. Here again the female assists the male in collecting the eggs and carrying them to the nest made of foam. In one case the fish retained the eggs in the mouth for the space of one minute, this being a matter worthy of particular note in this connection.

And last of all for the osphromenid fishes, we find an identical habit described in Zernecke's Leitfaden for 1907 in the male of *Macropodus viridi-auratus,* which is a cultivated variety of *Polyacanthus opercularis.*

There must now be noted certain isolated instances of mouth carrying by fishes, the last of which brings us close up to the gaff-topsail. In the year 1874 Carbonnier described the breeding habits of a fish exotic in France (having been imported from North America), which he called *Fundula cyprinodonta.* Dr. Theodore Gill, however, declared it to have been *Umbra pygmæa* (*Science*, Dec. 21, 1906). Carbonnier notes that after the extrusion and fertilization of the eggs "the female sucked them into her mouth and appeared to take great pleasure in rolling them around therein, nevertheless she replaced them" whence she had taken them. "When the eggs are found to to be a little scattered, it is clear that the female is no longer their only shield and protector, for the males also suck them into their mouths and pass them from one to another. At this time, the movements which they execute with their fins denote great satisfaction. As they replace the eggs without alteration and without damage, the female shows no air of inquietude; nevertheless at times she turns her head and makes pretense of driving them away." During hatching the female solicitously guards the eggs and carefully removes with her mouth all bad eggs.

One other instance, and we are ready to come to the catfishes. The miller's thumb, *Cottus gobio*, lays adhesive eggs which are guarded by the male. Of it Thomas Peek says (1869): "On the occasion of one of the small fish emerging from its egg, the parent chased it to the bottom of the tank, and, taking it gently in his mouth, replaced it in the cluster."

Further search through the literature would probably reveal other instances of this habit, but incidents enough have been given to make it clear that mouth transportation, as well as gestation, is not altogether unusual. However, similar habits are now to be described in members of the very family of catfishes themselves.

At the 1902 meeting of the American Association for the Advancement of Science, Dr. H. M. Smith, the present United States Commissioner of Fisheries, presented an interesting paper upon the breeding habits of the common yellow catfish, *Amieurus nebulosus*. The particular point in this paper which is of interest just here may be quoted as follows:

> "The most striking act in the care of the eggs was the sucking of the egg masses into the mouth and the blowing of them out with some force. The . . . . mouthing operations were continued with the fry until they swam freely, when the care of the young may be said to have ceased. . . . . The predaceous feeding habits of the old fish gradually overcame the parental instinct; the tendency to suck the fry into their mouths continued, and the inclination to spit them out diminished, so that the number of young dwindled daily."

The above is from the abstract of Smith's paper published in *Science* (Feb. 13, 1903). The complete account is to be found in a paper by Smith and Harron (1903), published in the Bulletin of the United States Fish Commission for 1902. In this they say:

> "The most striking act in the care of the eggs was the sucking of the egg masses into the mouth and the blowing of them out, this being repeated several times with each cluster before another lot was treated . . . .

"The very young fry were also taken into the mouths of the parents and blown out; especially those which became separated from the main lot and were found in sand and sediment. The old fish would take a mouthful of fry and foreign particles, retain them for a moment, and expel them with some force. After the young began to swim and became scattered, the parents continued to suck them in, and, as subsequently developed, did not always blow them out . . . .

"The fry which were left with their parents continued healthy, but their number steadily decreased. There being no way for them to escape; . . . it was suspected that the old fish were eating their young . . . . They were kept under close observation during the day, and were seen to be fond of mouthing the fry, more especially the weaker ones . . . . They were frequently seen to follow leisurely a fry, suck it into their mouth, retain it for a while, and then expel it, sometimes only to capture it again. There was no active pursuit of the fry, and the tendency seemed to be to spit them out. In one or two instances, however, it appeared that fry taken into the mouth were not liberated, the feeding instinct becoming paramount to the paternal instinct. After all the fry which had been left with their parents had disappeared, in about 6 weeks after hatching, 18 fry were placed in the aquarium one evening, and only 2 of these survived on the following morning."

It seems hardly necessary to argue the question as to the origin of the habit of oral gestation after the presentation of the facts above given. In the mind of the present writer there is no doubt that having begun by taking up the eggs and young for purposes of transportation, the fish have presently learned to retain them for longer and longer periods of time; we have a record of at least one minute's retention; and as the fish which retain their young even for short spaces of time and transport them to safer localities are more likely to leave descendants, through the action of natural selection, these fish and this habit will be perpetuated. Hence we may conjecture that as time has gone on the habit of retention has become more and more fixed until finally oral gestation has become an established habit.

Nor does the matter of abstinence from food offer any difficulty just here. It is known that many fishes which guard their nests do not feed while on such duty, and this is particularly true of the sticklebacks and gouramies. So this fits in well with the conjectural explanation just offered.

To the present writer all the data available lead to the conclusion just set forth, and to it he gives his full adherence.

LITERATURE CITED.

Becker, Karl. 1907. Der dreistliche Stickling (*Gasterosteus aculeatus*), Siene Zucht in Pflege in Zimmer Aquarium. Wochenschrift für Aquarien und Terrarienkunde, Braunschweig, Jahrg. 4, nr. 38.

Bleeker, Peter. 1858. Die Visschen van den Indischen Archipel. Ichthyologiæ Archipelagi Indici Prodomus, I, Siluri, p. 74.

Bloch, Marc Elieser. 1794. Naturgeschichte der Ausländische Fische. Achter Theil, p. 26, plate 365.

———, and J. G. Schneider. 1801. Systema ichthyologiæ. Iconibus CX illustratum. Post obitum auctoris inchoatum absolvit, correxit, interpolavit J. G. Schneider, p. 337, Berolini.

Boake, Bancroft. 1866. On the air-breathing fish of Ceylon. Jour. Ceylon Branch Royal Asiatic Society for 1865–1866, pp. 128–142.

Carbonnier, P. 1869. Sur le mode de reproduction d'une espèce de poissons de la Chine. Comptes Rendus Académie des Sciences de Paris, t. 69, pp. 489–491.

———. 1869^a. Rapport et observations sur accouplement d'une espèce de poisson. Bull. de la Société Impériale Zoologique d'Acclimatation, ser. 2, t. VI, pp. 408–414.

———. 1872. Le Macropode de Chine. Bull. Société Impériale Zoologique d'Acclimatation, ser. 2, t. 9, pp. 7–14.

———. 1874. Le Fondule (*Fundula cyprinodonta*). Bull. Mensuel Société d'Acclimatation, ser. 3, t. 1, pp. 665–671.

———. 1875. Nidification du poisson Arc-en-ciel. Compte Rendus Académie des Sciences de Paris, t. 81, p. 1138; also same title, Bull. Société Impériale Zoologique d'Acclimatation, ser. 3, t. III, pp. 11–22.

Clarke, S. C. 1883. American Angler, Dec. 15.

———. 1884. The fishes of the east coast of Florida. [in] Fishes of the East Atlantic coast that are caught with hook and line, by L. O. van Doren and S. C. Clarke, p. 145.

Coste, P. 1848. Nidification des Épinoches et des Épinochettes. Memoirs des Savants Étrangers à l'Académie des Sciences de l'Institut National de France, t. 10, pp. 586–587.

Day, Francis. 1873. On some new or little-known fishes of India. Proc. Zool. Soc. London, p. 705.

Eigenmann, C. H., and R. S. 1890. A revision of the South American *Nematognathi* or catfishes. Occasional Papers Cal. Acad. Sci., I, p. 36.

Evermann, B. W., and E. L. Goldsborough. 1902. A report on fishes collected in Mexico and Central America with notes and descriptions of five new species. Bull. U. S. Fish Commission, vol. XXI for 1901, pp. 140–146.

Goode, G. Brown. 1903. American fishes; etc., revised and largely extended by Theodore Gill, p. 379.

Green, John. 1858. Presentation of and remarks upon the breeding habits of several species of fishes from Surinam. Proc. Boston Soc. Nat. Hist., p. 328.

Gudger, E. W. 1912. Oral gestation in the gaff-topsail catfish, *Felichthys felis*. Abstract in Science, n.s., XXXV, p. 192.

———. 1916. The gaff-topsail (*Felichthys felis*), a sea catfish that carries its eggs in its mouth. Zoologica, Scientific Contributions New York Zoological Society, vol. II, No. 5, pp. 123–156. 12 figs.

Günther, A. C. L. 1864. Catalogue of fishes in the British Museum. Vol. v. p. 173.

Gurley, R. F. 1902. The habits of fishes. Amer. Jour. Psychol., vol. 13, p. 408–425.

Hancock, Albany. 1854. Observations on the nidification of *Gasterosteus aculeatus* and *G. spinachia*. Trans. Tyneside Naturalists' Field Club, vol. 2 for 1851–1854.

Hensel, Reinhold. 1870. Beiträge zur Kenntniss der Wirbelthiere Südbrasiliens. Archiv für Naturgeschichte, vol. 36, Bd. 1, pp. 50–91.

Hillhouse, William. 1825. Indian notices or sketches of the habits, characters, languages, etc., of the several nations of British Guiana, also the ichthyology of the fresh waters of the interior [of British Guiana], Demerara, 1825, p. 108.

Holder Charles Frederick. 1904. The boy anglers, p. 175. New York.

Houghton, William. 1865. Sticklebacks and other nest-making fish. Intellectual Observer, vol. VII, pp. 559–563.

Ihering, Hermann von. 1885. Die Lagoa dos Patos. Deutsche Geographische Blätter, Bd. 8, p. 106.

———. 1888. Ueber die Brutpflege und Entwicklung des Bagre (*Arius commersonii*). Biologische Centralblatt, Bd. 8, pp. 268–271.

———. 1896. Os peixes da costa do mar no Estado do Rio Grande do Sul. Annuario do Estado do Rio Grande do Sul, anno xiii, p. 115.

Peek, Thomas. 1869. Parental attachment of the Miller's thumb (*Cottus gobio*). The Student and Intellectual Observer, vol. iii, pp. 455–458.

Ransom, W. H. 1865. On the nest of the ten-spined stickleback. Annals and Mag. Nat. Hist., ser. 3, vol. 16, pp. 449–451.

Ryder, John A. 1883. Preliminary notice of the development and breeding habits of the Potomac catfish, *Ameiurus albidus*. Bull. U. S. Fish Comm., vol. iii for 1883, pp. 225–230.

———. 1887. On the development of osseous fishes, including marine and fresh-water forms. Report U. S. Fish Comm. for 1885, p. 533.

Smith, Hugh M. 1903. On the breeding habits of the yellow catfish. Science, n. s., vol. 17, pp. 243–244.

———. 1907. The fishes of North Carolina, p. 64. Raleigh.

———, and L. G. Harron. 1903. Breeding habits of the yellow catfish. Bull. U. S. Fish Comm. for 1902, pp. 151–154.

Steindachner, Franz. 1875. Ichthyologische Beitrage (IV): I. Zur Fischfauna von Panama. Sitzungsb. Kaiserl. Akad. Wissensch., Math. Naturwiss. Classe, Bd. 72, pp. 567, 575, 578.

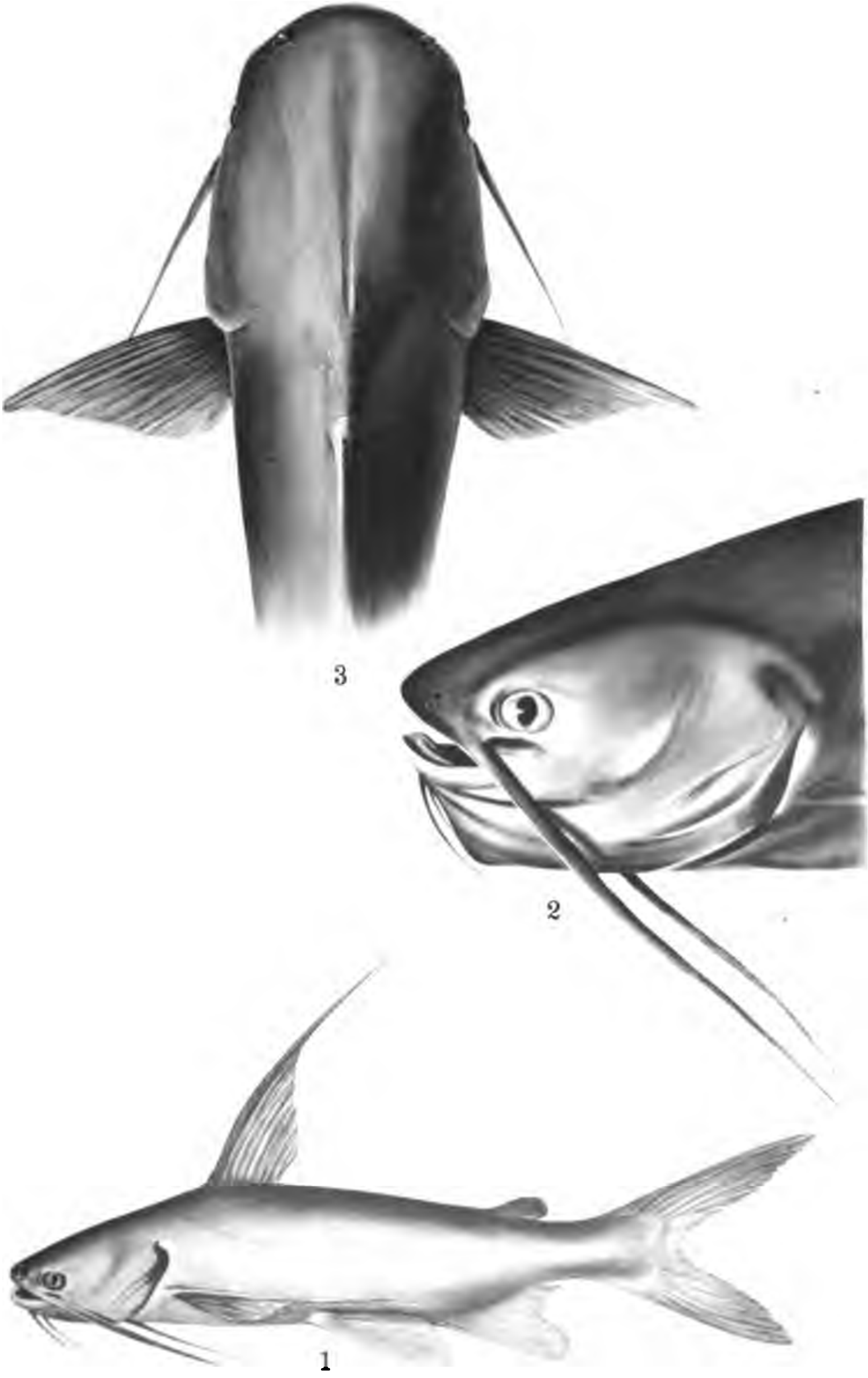
Turner, William. 1867. On a remarkable mode of gestation in an undescribed species of Arius. Jour. Anat. and Physiol. London, vol. i, pp. 78–82.

Waite, E. R. 1904. The breeding habits of the fighting fish (*Betta pugnax* Cantor). Records Australian Mus., vol. v, for 1903–1904., pp. 293–295.

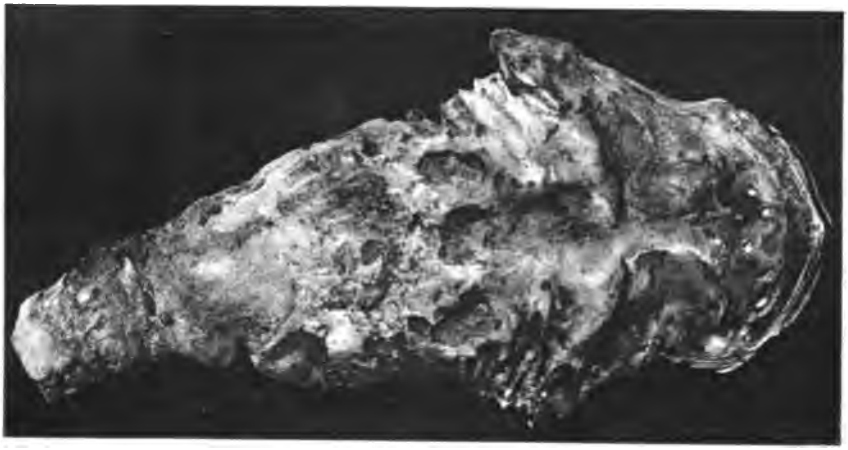
———. 1905. The breeding habits of the paradise fish (*Polyacanthus opercularis* L.). Records Australian Mus., vol. vi, 1905–1907, pp. 1–4.

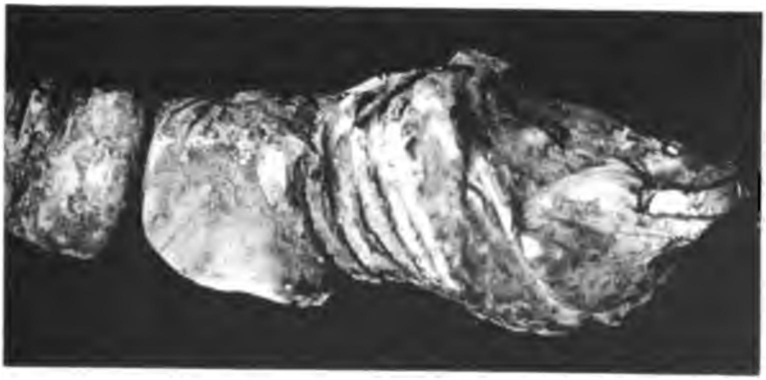
Warington, Robert. 1855. Observations on the habits of the stickleback (*Gasterosteus leiurus*). Annals and Mag. Nat. Hist. ser. 2, vol. xvi, pp. 330–332.

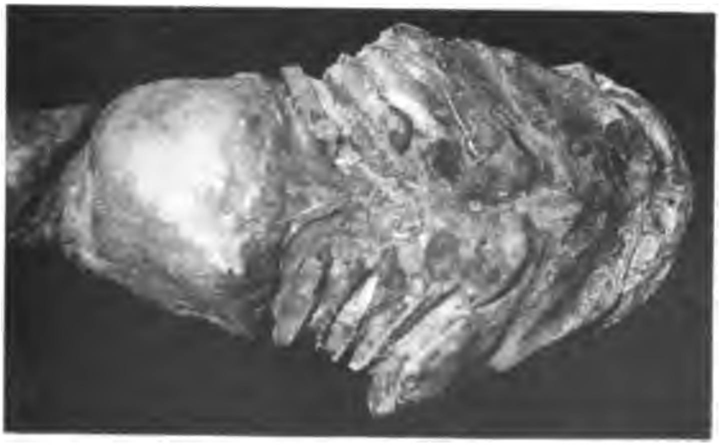
Wyman, Jeffries. 1859. On some unusual modes of gestation. Amer. Jour. Sci., ser. 2, vol. xxvii, pp. 5–13.



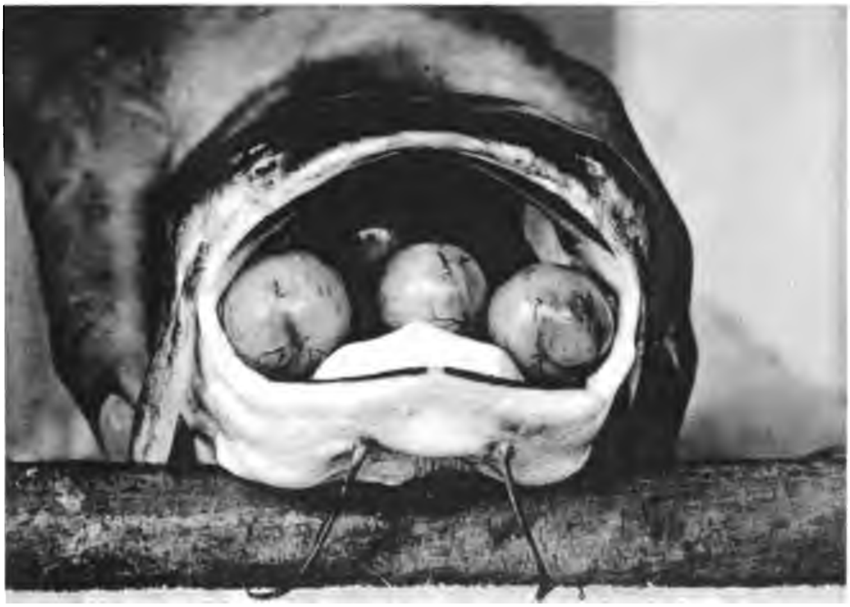
1. Lateral view of a female gaff-topsail, 19.25 inches long, drawn from life.
2. Lateral aspect of the head of an ovigerous male gaff-topsail showing the depressed hyoid region.
3. Dorsal view of head of the same fish showing the gill-covers distended to make room for the 36 eggs found in the mouth.


4

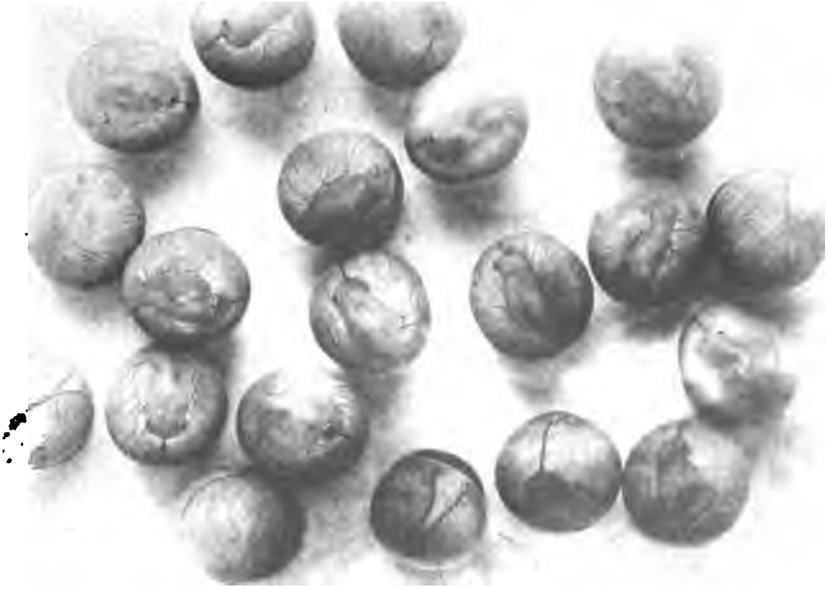

5


6

Dorsal, lateral, and ventral views of cast of mouth of male carrying 55 eggs.



7

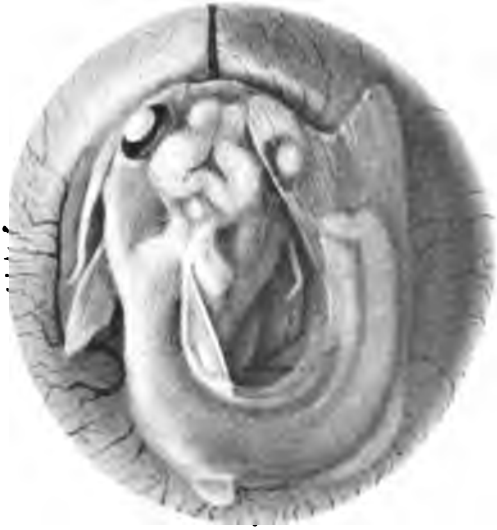


8

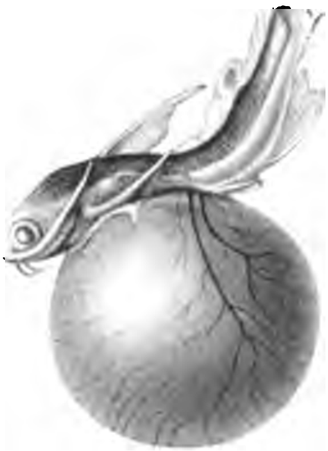
7. Front view of mouth of male *Felichthys felis* incubating eggs.

8. Live eggs of gaff-topsail showing the little fish with heads sunken in the yolk, tails curled to one side, body walls developing, and the extensive yolk vascular system.

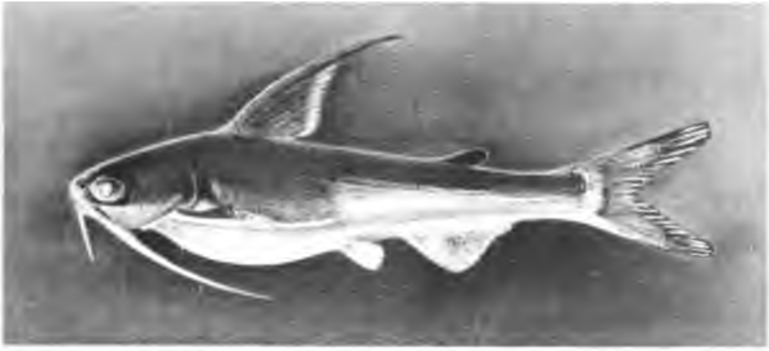
9



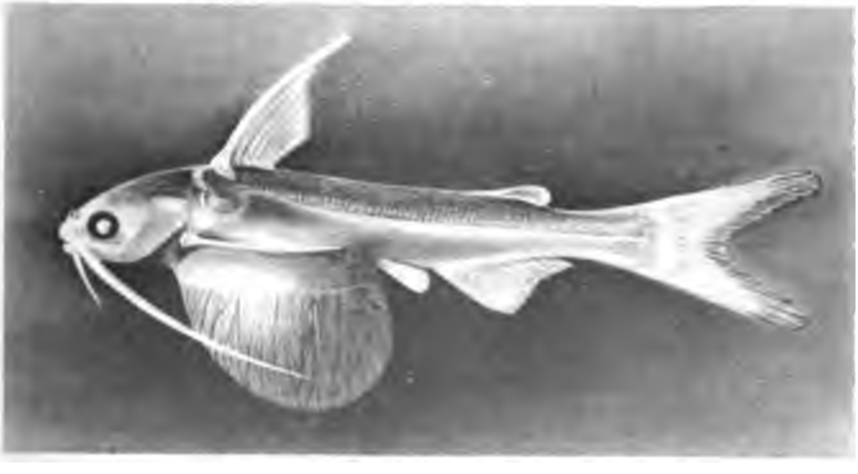
10



12



11



9. Young gaff-topsail about ready to burst shell, x 3.5. (Natural size of egg, 18.5 mm).
10 Young with tip-tilted tail shortly after being freed from the egg shell, x 1.75. (Natural size of egg, 20 mm).
11. Young in stage when "skating" over the bottom of the aquarium is the only method of progression, x 1.9. (Natural size, 58 mm).
12. Young in free-swimming stage with body walls completely closed over the yolk, x .88. (Natural size, 98 mm).

IV.

SPHYRÆNA BARRACUDA; ITS MORPHOLOGY, HABITS, AND HISTORY.

By E. W. GUDGER,

Of the State Normal College, Greensboro, North Carolina.

Seven plates, five text-figures.

CONTENTS.

	PAGE.
General Description	55
Color and Markings	56
Jaws and Teeth	59
Manner of Breathing	70
Internal Organs	70
Habits	74
How the Barracuda may be taken	77
Food and Feeding	79
Parasites	80
Use of the Barracuda as Food and Poisoning resulting therefrom	83
Size	93
The Barracuda Dangerous to Man	94
Habitat	99
Fossil Forms	100
Nomenclature	101
Drawings or Figures of the Barracuda	104
Literature Cited	107

SPHYRÆNA BARRACUDA; ITS MORPHOLOGY, HABITS, AND HISTORY.

By E. W. Gudger.

The family Sphyrænidæ, the barracudas, comprises one genus only, *Sphyræna*. The 20 or more species of this genus are found in the warm waters of the tropical and sub-tropical zones all round the world. They are carnivorous fishes, generally of large size, fierce and voracious in habits, the salt-water congeners of the fresh-water pikes.

GENERAL DESCRIPTION.

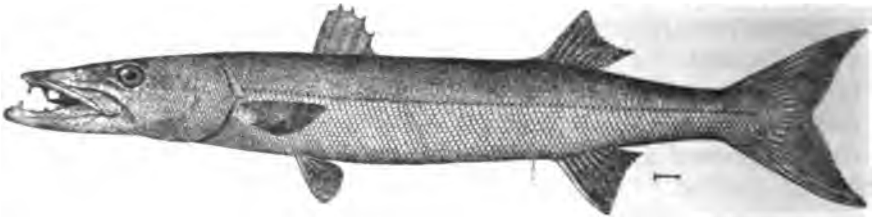
At Tortugas, the great barracuda, *Sphyræna barracuda* (Walbaum), also known in various parts of the West Indies as picuda or becuna, is very abundant in the lagoon. Twelve large specimens have been taken and examined, and scores of others could have been caught if they had been sought. This large fish has a long and powerful body, compressed and rounded, in cross-section having a somewhat elliptical outline. The head is very large, about one-fourth the total length of the fish, is long, narrow, pointed, and flat on top—in short, wedge-shaped. The mouth has an enormous gape, the jaws extending back to a point directly under the eye, and measuring about one-half the length of the head. The lower jaw projects beyond the upper, and both jaws are provided with huge canine teeth. The lower jaw, however, ends bluntly, as is shown in every figure of the fish given in this paper. In marked contradistinction are Fowler's figures (1903) of *S. picudilla* and *S. tome*, in both of which the lower jaw is drawn out into a fine thin point.

All of these points will be made clear by reference to figure 1, plate I, and figures 3 and 4, plate II, photographs of specimens taken at Tortugas and Miami, Florida.* Attention is also called to the spinous dorsal fin, which, with its five rays connected by a thin transparent membrane, is depressible in a groove, thereby becoming invisible. This fin is placed almost immediately over the abdominally located pelvic fins; while the soft dorsal, placed far back, occupies a similar position with reference to the anal fin. Not only are these two fins similar in position and appearance but also in structure, each being provided with a spine in front. The wide separation of the two dorsals and their location over the ventrally placed pectoral and anal fins gives the fish a symmetrical appearance despite its bulky figure. The body ends in a thick, stout caudal peduncle which bears the large, rather deeply forked caudal fin, of which the upper lobe is generally slightly the larger. The appearance of this fish gives one the idea of speed and power in about equal proportions. It is of the type of the battle cruiser.

---

*The figure on the opposite page (text-figure 1), which is reproduced from Frank T. Bullen's "Denizens of the Deep," represents the barracuda in thoroughly characteristic attitudes. The fishes are well drawn.

The body of the fish is well panoplied with fairly large cycloid scales. The eyes are large and staring, and are placed about the center of the upper lateral region. The anterior nostril is a small pore at the front end of the supraorbital ridge; the posterior is a slit (0.25 inch long in a 3 feet 10 inch specimen) standing nearly vertical (slightly forward and downward) in front of the eye. Our American authorities (Jordan and Evermann, and Evermann and Marsh) state that the lateral line is straight, but their figure shows that it rises slightly just above the pectoral fin. In my specimens, for the most part, this rise was far more marked than in their figure, in one fish beginning 2 inches back of the tip of the pectoral. In my largest specimen, however, the rise was only moderately pronounced.



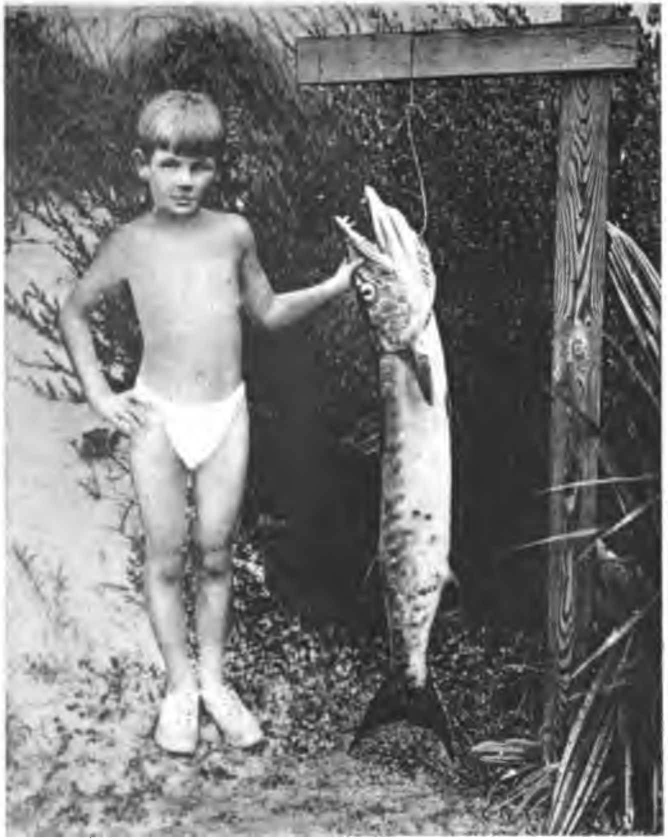
TEXT-FIGURE 2.—*Sphyræna barracuda* (Walbaum), after Jordan and Evermann.

As was noted above, 12 large specimens have been taken and examined, but the fish being so characteristic in structure and markings that it is recognized at a mere glance, fin ray count was made for only one specimen. This fish, an adult, 3 feet 10 inches in length, had dorsal rays V–I, 9; and, I, 8. Jordan and Evermann (1896) give D. V–I, 9; A. I, 9; but their figure has only 8 anal rays. Evermann and Marsh, in their "Fishes of Porto Rico" (1900), make the dorsal rays V–I, 10; and the anal ones I, 8; yet they copy Jordan and Evermann's figure referred to, which has but 9 soft rays in the dorsal. Possibly there is some variation in the number of fin rays. The figure referred to is reproduced herein as text-figure 2.

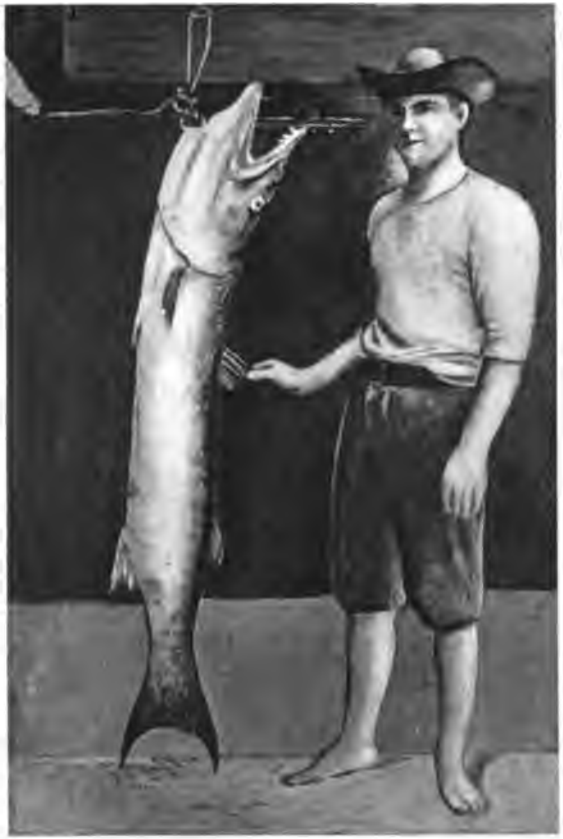
The table on page 58 gives the comparative measurements of these 12 specimens in inches and the weight in pounds.

COLOR AND MARKINGS.

There is considerable variation in the body-color and markings of this big barracuda. In life, specimen No. 11 was on the upper surface a rich dark green, the sides silvery, the belly chalky-white. In the mid-lateral region the green sent down bars into the silvery region,



1



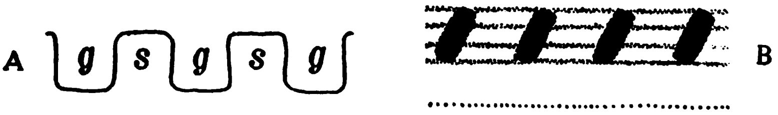
2

1. *Sphyræna barracuda* and eleven year old boy, Tortugas, 1912.
2. Barracuda from Cocos-Keeling Archipelago, after Wood-Jones, 1902.

which in turn sent white bars up into the green, the two forming an interlocking or dove-tailing, as shown in the diagrammatic figure (text-figure 3 A). Unfortunately, the number of these bars was not counted. Specimen No. 10 (3 feet 10 inches long), shortly after being killed, was dorsally black with an iridescent metallic blue in some lights; it had some blue on the sides, lower down was silvery, and the ventral parts were a chalky-white.

Evermann and Marsh (1900) speak of "dark longitudinal streaks along rows of scales above lateral line." This was confirmed on one fish only. Of No. 1, my notes say, "faint dark longitudinal stripes or streaks above lateral line." This was my smallest specimen, 2 feet 1.5 inches in extreme length. The largest Porto Rican specimen was 16 inches long. It seems probable that this is a juvenile marking which disappears with age.[1]

Reference has already been made to the bars found just above the lateral line on each side of fish No. 11. On fish No. 1, lying wholly above the lateral line and crossing the horizontal streaks at a large acute angle forward, were 18 or 20 dark bars as shown in text-figure 3 B. No. 5 had 18 bars, as noted for No. 1, but no longitudinal streaks were visible. No. 7 had about 18 similar bars, as did No. 8. No. 9 had about 20. No. 10 had 17 on the right side and 18 on the left. No. 11 has already been referred to, and the last and longest specimen either had no such bars or I failed to note them. In the case of No. 6 these notes explicitly state that the bars were lacking. In addition to these bars or oblong more or less rectangular blotches found on the body forward of the dorsal-anal fin region, there were generally present on the lateral hinder parts certain interesting spots to which attention will now be called.



TEXT-FIGURE 3.

A.—Dove-tailing of the upper green and the lower silvery colors on the side of barracuda No. 11. (Semi-diagrammatic).

B.—Longitudinal stripes and vertical bars on right side of barracuda No. 1.

No. 4 had on the hinder half of the right side of the body 15 large black spots, some faint, and some apparently of two run together; but on the left 9 spots only. No. 9, in addition to the 20 black bars above the lateral line on each side, had below the lateral line 23 black spots, some faint and some apparently double. On the left side there were 18. All were mainly behind the anterior margin of the second dorsal. No. 10 had 18 black spots on the right and 14 on the left side. These were below the lateral line and extended from a point just anterior to the anal back to the caudal peduncle and on this some were found above the lateral line also.

---

[1] Some months after writing the above, Professor W. H. Longley informed me that on July 14, 1916, there was taken out in open water at Tortugas, a little barracuda "about an inch long which was marked with a distinct lateral band of brown pigment running the length of the body through the eye."

No. 12 had on the right side about 15 spots; some large, some small: some distinct, some faint. On the left side there were 2 large spots about amidships, and behind these about 6 small indistinct spots. Furthermore, interesting to note, there were on the ventral surface of the body, on the median line, between the anal and caudal fins, quite a number of dark spots. These were not noticed on any other fish.

Evermann and Marsh (1900) speak of "usually from one to several small, very dark brown spots, sometimes black, scattered irregularly on the side." They also say that the young have dark irregularly shaped blotches or bars sometimes disappearing with age. However, from a study of my specimens described above it would seem that this conclusion does not apply to the Tortugas form. Further it is interesting to note that No. 2 (2 feet 4 inches in extreme length) had but 4 spots on the right and 3 on the left side, some of these being very pale. Then again No. 6 (3 feet 1.5 inches outside measurements) had no spots at all. This may have been *S. guachancho*, which is described as being devoid of any color markings whatever, lacking "regular bars and scattered spots." In this connection it is of interest to note that Temminck and Schlegel (1850) in writing of Japanese barracudas say that the young of *S. vulgaris* have the upper parts covered with brownish marblings and that on the lateral line there is often found a range of dark spots, oblong and rather closely crowded.

Measurements in inches.	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 12
Length to—												
tip of caudal	25.5	29	30.5	34.3	35	37.5	40	40.8	43	46	49	55
fork of caudal				32		34.5			40		46	52.8
base of caudal				29	30	32	34	35.5	36	38	39	47
anterior base D. II										29		
Length of head			8	9		9.5				12.5	13	15
jaw												7.5
spine 2 of D. I												3.9
Girth back of pectorals		9.5							14	15		20.5
at front edge D. I	9			11	12	12.8		14		14	16	22.5
at base of caudal										7		8
Depth		3.8	4	4								8.8
Diameter of eye			1	1		1			1	$\frac{7}{8}$ x $\frac{9}{8}$	1	1.5
pupil										$\frac{9}{16}$		
Spread of pectorals										11.5		
D. II and anal										11		
caudal							10			13		
Weight—pounds	2.5	4		6		9	9		13	17.5	21	38

The following are descriptions of the markings of four small barracudas taken at Tortugas in the season of 1917, which were kindly sent to me by Dr. A. G. Mayer. In transit the bottle containing them was broken and they reached me in a dried condition, but with their color markings quite clear and well defined. No. 1, measuring 2.6 inches from tip of snout to base of caudal, has, between that point and the hinder edge of the opercle, about 9 dark blotches which extend across the dorsum and down to the belly on each side. Another fish of the same length, but hardly so bulky, has about 7 of these saddle-shaped bands. A third, 2.35 inches to the base of the caudal, has about 6. The last and smallest (2.25 inches) has 7 bands, possibly the most definitely marked off of all. In addition to these bands noted, there is another, not quite so clear, extending from the fore part of one opercle across the back to the other gill-cover. Again, on the dorsum, across the base of the skull, all four specimens show a dark blotch.

These bands are especially large and are somewhat irregular in shape in the region of the lateral line on each side. On the median line of the dorsum, especially in the region of the second dorsal fin, these bands run together, giving these parts a very dark, even black appearance. There can be no doubt that, as the fish grows older, the dorsal connection disappears and the lateral parts of the bands are left as the dark bars or splotches described above and shown in figure 1, plate I.

These colors referred to above were noted on my fish after death. In life the color of the fish readily accommodates itself to its surroundings. A large barracuda basking near the surface of the water in the neighborhood of a coral head, a buoy or a channel stake, appears much as a ghost fish, a shadowy wraith. Another lying near the bottom over coral sand will so accommodate itself to its environment as to be almost unnoticeable so long as it remains quiet. Wood-Jones (1912) says that it is the hardest of all sea fish to see.

JAWS AND TEETH.[1]

The mouth parts of the barracuda deserve attention both for themselves and because they do not seem to have been adequately described. The mouth is very large, being in length nearly half of the head and having a large gape. Together with this, the projecting lower jaw, the enormous canine teeth, the large glassy staring eyes, give the fish an aspect of ferocity which is not belied by its habits, as will be shown later. It has been fittingly compared to a bull-dog, and Saville-Kent (1893) notes that at Moreton Bay, Australia, it is called *dingo* because of "its formidable array of teeth."

The lower jaw is longer than the upper, projecting notably as a conical fleshy mass of tissue which, if on the upper jaw, would form a decided snout. Dissection of this jaw in the fresh specimen revealed the following interesting structure: The rami of the mandible are as usual united at the apex by a mass of cartilage, but what is unusual is that this cartilage projects forward as a considerable lump which, covered by flesh and skin, gives the lower jaw the appearance shown in figures 3 and 4, plate II, and figures 7 and 8, plate III. For the dissected jaw see figure 13, plate IV. It should be noted, however, that in cutting away the flesh and skin some of the cartilage was unwittingly removed, hence the projecting mass is not as great as in nature. This conical lump forms the terminal part of the rather thin fibrous lip of the lower jaw. This lip is freely movable and has, fastening it to the skin of the jaw, a number of short oblique backwardly trending slips of tissue. These allow free motion of this lip. For these structures see figures 4 and 8, plates II and III.

---

[1] The material on which I have based my observations for this section consists of four dried heads from Tortugas (dried with the mouths distended) and one fresh head sent to me packed in salt from Miami, Florida. For this last specimen and for previous similar favors I am indebted to Mr. John Mills, of Miami.

Turning now to the upper jaw, it is readily seen that the premaxillaries lie outside of and are separated from the maxillaries by a deep groove. In front, the symphysis of these bones is, like that of the lower jaw, covered by a mass of soft tissue. This mass, however, is much smaller than that of the lower jaw, does not form such a conical projection, and is comparatively thin and upturned. At the very point on the median line this is hollowed out into a deep pocket for the reception of the great fang of the lower jaw. To aid in the formation of this cavity, the terminal portions of both premaxillaries are hollowed out to the point of union to form a little bay. Viewed dorsally, the maxillaries seem to lie above and (in the hinder region) outside of the premaxillaries, but in front they swing toward the median region. Viewed from below, the true situation is revealed; posteriorly they lie outside the premaxillaries, but about one-fourth the distance forward they cross over these bones and come to lie inside and are united by tissue in the region of the hinder group of great fangs on the premaxillaries. Attached to each premaxillary is a thin fold of skin forming a less pronounced and less movable lip than is found on the lower jaw. These structures are seen in figures 7, plate III; and 11 and 12, plate IV.

The articulation of the upper jaw to the skull is entirely unique and hence worthy of description. The premaxillaries are non-protractile and are almost incapable of motion. The maxillaries, however, are so hinged onto the anterior end of the cranium as to allow the tip of the upper jaw to be raised in a vertical plane. This interesting articulation is shown in figures 11 and 12, plate IV. The vomer has each shoulder beveled off dorsally and anteriorly, and in front there is a stout spine rounded off on the upper surface and the anterior end. The palatines send forward stout processes rounded in front, flattened below and especially on the inner edges. Between these two processes above and the projecting point of the vomer below there is seen a considerable space, if one looks at the skull laterally. In a ventral view the maxillaries are seen to have just behind their symphysis a pair of pockets or recesses; a dorsal one as wide as long, and a shallow ventral one lying anterior to the other. The roof of the larger recess is made by the nasals, which are united by and imbedded in a mass of cartilaginous material. The sides of this pocket are formed by two rounded backwardly and slightly inwardly projecting prominences of the maxillaries. Viewed from above, the anterior ends of the maxillaries, just outside the processes described, are hollowed out into shallow cavities whose use will be described below. Figures 11 and 12, plate IV, show these structures both from below and from above.

In articulation these bones fit together as follows: the forward prolongation of the vomer fits into the posterior and dorsally placed cavity between the maxillaries; the backwardly projecting processes of the premaxillaries fit into the spaces between the shoulders of the vomer and the forwardly projecting processes of the palatines; while the united nasals lie dorsal to the frontal process of the vomer and between the forwardly projecting horns of the palatines; and lastly the flattened and rounded ventral surfaces of these same horns fit into the grooves excavated into the anterior dorsal surfaces of the maxillaries. Thus there is formed a beautiful joint allowing little motion laterally but considerable in the vertical plane. However, the union of the right and left halves of both maxillaries and premaxillaries by ligaments and cartilage allows a widening of the upper jaw at the angle of the mouth, as will be shown below.

The rami of the lower jaw are likewise united in front by cartilage and connective tissue, and behind they are hinged onto the quadrates. These in turn are immovably attached by suture joints to the palatines. These latter bones are hinged like a door or the lid to a chest, posteriorly to the prefrontals and anteriorly to lateral projections of the vomer. In widening the mouth, the upper and lower jaws spread apart behind and the quadrates and palatines swing outward and upward. This takes place at the same time that the lower jaw drops downward while the tip of the upper jaw is raised. All this results in giving the barracuda an enormous gape, a necessity for a fish which preys on other large fish and which swallows its food in large fragments. These points are well shown in figures 5, plate II; 7, plate III; 11, 12, and 13, plate IV.

Specimen.	Left.	Right.
Fresh........	13+7	11+4+3e
Dried No. I...	11+5	12+2
II..	13+4	11+3
III.	14+3	16+5
IV.	17+4	12+6

The lower jaw is filled with knife-like canine teeth set palisade fashion just inside the lip-like membrane. These are small in front but larger in the middle and posterior parts. They point slightly backward, especially those in rear. Their numbers for my five specimens appear in the accompanying table, the dried heads being taken in the ascending order of size. The + teeth are the small ones at the front of each ramus of the jaw; those marked +e are in the act of erupting. Two factors seem to account for this variability. First the size of the fish; the larger the fish the greater the number of teeth. The fresh head was unfortunately not measured, but a study of the teeth and comparative measurements of the bones show that it was about the size of the dried head No. III, and possibly a little larger. The second factor relates to the number of teeth that have been broken off without sufficient time having elapsed for their being replaced. This is particularly true of dried head No. IV, where on the right side several teeth have been broken off. Figure 13, plate IV, from a photograph of the lower jaw of the fresh specimen, will make these points clear.

In front, at the symphysis of the rami of the lower jaw, is a huge fang, bowed on the front edge and slightly hooked backward. This was single in each of my five specimens, on the right side in four specimens and on the left in one. Two of these teeth might be expected but one seems to be always broken off. This matter will be discussed later, but it should be noted here that this tooth is always inclined toward the vertical median plane of the head and jaws—that on the left side towards the right and that in the right *vice versa.*

The upper jaw has in front at the apex, under the snout, a group of huge knife-shaped fangs, two, three, or four on each side, not two pairs as Jordan and Evermann state. The two anterior of these generally point about straight downward, while the posterior ones are sharply hooked backwards. All are convexly bowed on the front edge. These teeth may be seen in nearly every figure of the fish and of its head given in this paper. The accompanying table shows their numbers in my five specimens.

Specimen.	Left.	Right.
Fresh..........	3	2+3e
Dried No. I....	2	2
II...	2	2
III..	2+1e	2
IV..	3+1e	2+1e

Implanted on the palatines in palisade fashion are a number of large, pointed, lancet-shaped teeth, in structure and arrangement very similar to the mandibular teeth but much larger. These are largest in front and decrease in size backward, becoming very small at the rear. The most anterior ones are but little if any smaller than the great fangs and on each side the series runs backward in line with these fangs. These teeth have, however, no physical connection with each other, there being a wide hiatus between them, as may be seen in figure 12, plate IV. The accompanying table gives the numbers of these teeth for each specimen. The + teeth are the small ones at the rear, while +e indicates teeth just erupting. Here again, it will be noted that the larger the specimen the larger and more numerous the palatine teeth.

Specimen.	Left.	Right.
Fresh..........	6+2+2e	4+1+1e
Dried No. I....	4+2	5+3
II...	7	6+1
III..	7+2	9+2
IV..	10	8+1

Closely set in palisade fashion, beginning about opposite the space between the first and second great fangs, are the small knife-like teeth of the premaxillaries. These teeth, pointing slightly backward and extending clear to the angle of the mouth, give the upper jaw a very saw-like appearance, as may be seen in figure 8, plate III. The data tabulated herewith would seem to show that the number of these teeth is fairly constant, the discrepancies being chiefly due to teeth broken off and not yet replaced.

Specimen.	Left.	Right.
Fresh.........	30	44
Dried No. I....	45	34
II...	41	29
III..	41	47
IV..	38	47

An interesting point was passed over in the dried heads and seen only when the fresh head was dissected—that the teeth of the lower jaw, those of the premaxillaries, those on the palatines, and even the great fangs under the snout; all the teeth in the mouth, except the solitary fang of the lower jaw, are set "overlapping." That is, in every case, where the teeth are crowded, the front edge of every tooth rests on the outer side of the tooth before it, and its hinder edge is set on the inside of the tooth behind it; going from *behind forward,* the teeth over-lap like the shingles on a roof. This is especially apparent where new teeth are ready to erupt. Reference to figures 12 and 13 on plate IV, photographs of the cleaned-off jaws, will make this plain.

When the mouth is closed, the parts of this formidable dental apparatus are approximated sausage-mill fashion, as follows: The teeth of the lower jaw fit into deep grooves between the premaxillaries and palatines of the upper jaw and the big anterior canine fits into the pocket of the upper jaw which has already been described; the premaxillary teeth of the upper jaw come to rest against the lip-like membrane lying just outside the great teeth of the lower jaw, while the huge palatines fit into a wide, deep space between the lower jaw and the tongue, and finally the big anterior canines are received into special recesses in the membrane lining the anterior part of the floor of the mouth just behind the symphysis of the mandibles. Held in such teeth no fish can escape save by leaving part of itself behind.

The sausage-mill approximation of these teeth will be clearer after an examination of figures 6, plate II; 12, plate IV; 9 and 10, plate III. The particular point to be noted is the presence of the mandibular breathing valve, and in its forward part the depressions in it which accommodate the great canines of the upper jaw. However, the modification of the mouth parts to fit these huge teeth does not end here, but extends to the very bones themselves. In figure 13, plate IV, the reader will discern on the inside of each ramus of the lower jaw just behind the junction an excavation in the bone. These are to receive the points of the great canines of the upper jaw.

There are no teeth on the vomer, but on the tongue is a number of rows of small, sharp-pointed recurved teeth which must materially aid in the swallowing process, as do the large number of small teeth covering the pharyngeals above and below and the inner edges of the gill-arches. These are quite necessary, since the great barracuda chops its prey (if of any size) into large fragments which are swallowed whole. In addition to the structures described, there is a large breathing valve in the upper jaw, just posterior to the four great canines and anterior to the palatine teeth. Below, a similar structure is found just in front of the tongue. The tongue has considerable freedom of motion.

On the under side of the lower jaw, just behind the point of junction of the mandibles, are two round apertures leading into blind sacs extending both backward and forward some little distance. What function these subserve is not known to the writer. They are shown in figure 6, plate II.

Before leaving this subject it may be of interest to give the sizes of some of these great teeth. In fish No. 10 (3 feet 10 inches long), the single tooth at the apex of the lower jaw measures 13 mm., the big upper front teeth are 15 mm. long free of the gum, and the knife-blade teeth implanted on the palatines run from 9 to 13 mm. The large teeth of specimen No. 12 (55 inches long), which is No. IV of the tables of teeth, have for the most part had their points broken off and hence their measurements, generally speaking, do not show up so large as they should. Thus the big lower tooth measures only 11 mm., while the upper ones run from 12 to 14 (tips broken) to 17 and 18 mm. (perfect teeth). The palatine teeth of this fish run from 9 to 16 mm. These measurements, it must be understood, are all made from dried specimens and may possibly be slightly greater than in the freshly caught fish. When the flesh was cleaned off of the fresh head, on the lower jaw the big anterior tooth (tip broken) measured 10 mm. and the great chopping teeth further back ran from 6 to 9 mm., averaging about 7 to 8 mm. The great fangs of the anterior upper jaw measured 11, 13 (two teeth), and 14 mm. (Two other teeth were at the point of erupting.) The four great incisors on the right palatine measured 12, 14, 8 (tip broken), and 10 mm. from before backward. Similar measurements for the four corresponding teeth on the left palatine were 12, 11, 11, and 9 mm.

Figure 5, plate II, shows the number and relative position of these great teeth of the upper jaw. Some idea also can be gotten of the size, shape, and position of those of the lower jaw. The photograph was taken looking into the mouth from behind. The two inner groups of teeth are the great fangs on the premaxillaries. The outer groups are the huge knife-shaped teeth on the palatines.

Inspection of figures 12 and 13, plate IV, shows that the teeth are set in alveoli or sockets in each ramus of the mandible and the premaxillary, and in each palatine. These teeth are polyphydont—that is, they are replaced by new teeth as soon as they are worn down, broken off, or become loosened in their sockets. In some socketed teeth the succession is vertical, *i. e.*, the new tooth is formed in the same socket as the old one and grows out at the same aperture. This is by virtue of the fact that the base of the old tooth is absorbed and the new tooth thus comes to lie below it and to take its place. In other fishes the new tooth succeeds its predecessor, growing side by side with it or its stump.

In the great barracuda the teeth are set in sockets, out of which they grow by multiplication of cells at their pulpy bases. Room for the new teeth is often made by the old teeth being broken off, whereupon the root is resorbed and a new tooth erupts alongside the first to take its place. This state of things can be seen in the photographs of the skull of the specimen from Miami, as shown in figures 12 and 13, plate IV. In the figure of the lower jaw, the fang at the terminus of the left ramus has been broken off and already one sees the chink through which the new tooth will emerge. In figure 12, of the upper jaw, we have a similar state of affairs. The left front fang has been broken off, the one next to it has become loose in its socket and in the space between the two is seen the hole out of which the new tooth will come.

Closer inspection of figures 12 and 13, plate IV, will show some two dozen similar cases among the premaxillary, mandibular, and palatine teeth. All stages can be made out from the recently broken-off stumps to the half-grown replacing tooth. This is the reason for the variable number of great fangs at the apex of the upper jaw. Some of these in the cleaned head, it should be noted, were so loose in their sockets that they had to be glued in to prevent their being lost. A similar state of affairs is revealed by a close examination of the jaws of the three dried heads. The study of the succession of teeth in this fish would be of both interest and value. It would, however, have to be undertaken where there was an abundance of fresh material.

Before taking up the literature of this phase of the subject it is well to emphasize the fact that all my specimens and their figures show but one fang at the symphysis of the lower jaw. For this see the figures of the dried heads on plate III; and figure 13, plate IV, showing the teeth of the fresh specimen from Miami. These single fangs are always inclined toward the median line, the ones situated on the right side are inclined towards the left, and *vice versa.* Inspection of the largest dried head and of the fresh specimen, however, shows that the left tooth has only recently been broken off; while in dried head No. III, a new fang is just ready to erupt on the right side. Apparently two teeth belong at the apex of the lower jaw, but only one seems to be present at any given time. This point will be taken up again after the literature of the subject has been reviewed.

The earliest writer whom I have found to give any accurate description of the jaws and teeth of *Sphyræna* is Rondelet (1558).* Writing of the European form, the spet, he says:

> "The lower jaw is longer than the upper, ends in a point, and receives the upper jaw into itself. . . . It has strong pointed teeth curved inwards like those of the Muræna. On the upper jaw there are four, and on the palate are two rows of teeth. At the center (apex) of the lower jaw there is one tooth greater than all the others, which enters into a cavity in the center of the upper jaw made on purpose to receive it."

Even clearer and more explicit is the Roman physician, Salviani, whose folio work on fishes appeared in the same year as that of the Latin edition of his contemporary. He writes:

> "The upper jaw is obtuse and shorter, the lower acute and longer. As a result, the point of the wedge-shaped beak is constituted only of the extreme part of the lower jaw; and into the lower and longer maxilla, the shorter upper jaw fits when the mouth is closed. Each jaw is fortified with teeth, the upper with larger ones, the lower with smaller; and in the middle of the anterior part of this lower jaw, indeed in the very hiatus of the mouth, there stands out one tooth the longest and sharpest of all. This, when the mouth is closed, is so received into a foramen in the anterior and membranous part of the upper jaw, that this projects a little on the outside."†

These descriptions, though made from the Mediterranean species, are more accurate for our fish than is Sloane's, even though his was written from the West Indian form found at Jamaica. Sloane says (1707), of a barracuda 15 inches long and hence immature:

> "The under jaw had two Rowes of small Teeth, and one long one at the End in the Middle, the upper had one Row of small teeth on the outside and another within of long ones."

Catesby's description (1754) is also very imperfect. For his fish see figure 16, plate V. He simply says of Bahama specimens:

> "The upper jaw is armed with four large teeth, placed at [opposite to] the fore part of the under jaw; next the head are placed ten smaller teeth, being five on each side; and in the fore part of [the lower jaw] . . . grows one single large tooth."

Patrick Browne (1756) found two large canines at the apex of the lower jaw, which he notes was the longer. The mouth is described as very large, the jaws especially so. These are filled with many oblong lanceolate teeth, "whereof the two foremost [on the lower jaw] pierce through so many sockets formed in the tips of the upper jaw, while others lodge on either side of the opposite teeth." He seems to be the first to note the presence of teeth on the tongue and of the two lower fangs.

---

*The first edition of Rondelet's work was published in Latin in 1554.

†This is faintly shown in Salviani's excellent figure.

Fermin (1769) contents himself with saying that the jaws are filled with long and trenchant teeth which nothing can withstand.

Parra's interesting book on Cuban fishes, published in 1783, contains a pretty fair description of the dental apparatus of the West Indian barracuda, perhaps the best up to date. His figure, however, is crude. See figure 17, plate v.

His statement reads:

> "In the upper jaw these fish have in front four teeth larger than the others, and posteriorly three or four others also large: on the external lip of this jaw they have a row of teeth small in size. In the lower jaw they have one in the middle, also large, which corresponds to a space which has been noted in the upper jaw. . . . there appear on the margin of the lower jaw, teeth small at first but which soon grow larger and articulate with the large and small teeth of the upper jaw. To their sharp edges and position as described is due the fact that these animals cut like a knife when they bite."

The most accurate description of the jaws and teeth of fishes of the genus *Sphyræna* that I have seen is that by Cuvier and Valenciennes, in the third volume of their *Histoire naturelle des poissons* (1829). In speaking of the Mediterranean form, *S. vulgaris*, the common spet, they note that the lower jaw ends in a fleshy point, that the tip of the upper is truncated to adapt itself to the curve of the lower, and that both have membranous lips outside the teeth. Cuvier and Valenciennes's description of the jaws was not read until months after my own specimens had been described, yet the reader of this paper will find the descriptions almost interchangeable. They say:

> "The intermaxillaries [premaxillaries?] have along their edge a single row of very small teeth, numerous and sawlike; but at their anterior extremity and a little inside, they each have two large ones, one behind the other, compressed, trenchant, a little arched and pointed. A little further back and at some distance from the intermaxillary teeth, but in the same line, there are on each palatine three or four equally large, cutting and pointed, but not arched teeth; then there follow backward along the length of each palatine twelve to fifteen other teeth, very small and shaped like saw teeth, after the fashion of those on the intermaxillaries.
>
> "On the lower jaw there are two strong cutting teeth, pointed and hooked, which correspond in this anterior region to the four on the upper jaw. The fish often has only one and this gives it the air of never having had but one at the end of the lower jaw. Along each side of this jaw one sees at first sight a series of a score of very small teeth of which the hinder ones become very large and trenchant but not half equalling the great palatine teeth which are directly above them. When the mouth is closed the lateral teeth of the lower jaw enter into the interval between the intermaxillary and palatine teeth of the upper. The vomer has no teeth."

Of the great barracuda, Cuvier and Valenciennes give an excellent colored figure accompanied by a line drawing of the jaws and teeth. The former figure is reproduced as figure 18 of plate v, while figures 19 and 20, plate vi, show both the line drawing referred to and the head of the colored figure. The reader is particularly requested to contrast the figure of the jaws and teeth with the photographs of Florida specimens. Cuvier and Valenciennes note that the great teeth are very large and non-arched, but their figure has them (especially the upper ones) curved backward. Of the other teeth they say:

> "Each palatine has a number of large teeth, which may range from five or six to ten or twelve, without any little ones, either in their intervals or farther back. At the most each has three or four small ones, which are only seen in young specimens or when one cleans off a skeleton. Hence the edge of this bone is sharp and smooth."

In their full-length figure the great teeth above and below are hooked backwardly; there is only *one* big canine below but two above. In the line drawing two lower canines are seen straight and unpointed, while the four big upper ones are backwardly hooked.

Louis Agassiz (1843) not only figures the jaws and teeth of *S. barracuda* but the skull and skeleton as well. His beautiful figure, which in the main points is technically correct, is herein reproduced as figure 21, plate vi, while figure 22, plate vi, is an enlarged photographic copy of the skull and adjacent parts. The one point criticizable is that the pelvic fin is possibly placed slightly too far forwards. After noting that all the parts of the head are much elongated Agassiz says:

> "The intermaxillaries have a single row of small teeth on their lateral border, but in front and a little inside there are two very large ones, compressed and very trenchant, slightly bowed and very pointed, accompanied sometimes by one or by two teeth much smaller. Farther back and on the same line, the palatines bear a series of five or six equally large, sharp and pointed teeth, not bent but for the most part like lance heads. Succeeding also to these on the length of the palatines are twelve or fifteen teeth much smaller and serrated like those on the intermaxillaries. The lower jaw has only two large trenchant teeth, pointed and bent, at the anterior symphysis of the branches of the mandible, which correspond to those of the upper jaw. Along each arm of the lower jaw, there follows each other a series of teeth, trenchant, straight, pyramidal in appearance, more or less large and more or less elongated. These correspond to the grand palatines and fit in between these and the intermaxillaries when the animal closes its formidable mouth."

To recapitulate, Rondelet (1558) and Salviani (1554) each found a single tooth at the symphysis of the branches of the lower jaw; so did Sloane (1725) and Catesby (1754) and Parra (1787), while Büttikofer (1890) found but one in the lower jaw of *S. jello* caught off the coast of Liberia. Jordan and Evermann (1896) call for and figure but one. Fowler (1903) found but one in *S. tome*. Bullen (1904), in his very spirited figure (text-figure 1), shows but one fang. Wood-Jones (1912), in his photograph (figure 2, plate I) of a giant specimen from Cocos-Keeling Islands, shows but one tooth. My five specimens, as their photographs show, had but one fang each. On the other hand Browne (1756) speaks of two teeth at the symphysis of the lower jaw as does Guichenot in his "Poissons de Cuba" (1850). In describing the spet of Mediterranean waters, Cuvier and Valenciennes (1829) distinctly say that at the point of the lower jaw are two large, strong, curved teeth. Of the great barracuda they say that the dentition is like that of the spet. Their full-length figure of *S. barracuda* shows but a single tooth at the apex of the lower jaw, but their line drawing of the head found on the same plate shows two straight fine-pointed teeth. The single tooth in the other figure is, however, correctly hooked backward. Agassiz (1843) likewise says that the lower jaw has two great teeth at its apex, but his figure shows only one. (See figure 22, plate VI.) The last to be quoted is Day (1865), who says that there are two large canines in the anterior part of the lower jaw of *S. jello* of India.

It is interesting just here to note that Cuvier and Valenciennes quote Plée as saying that he has seen great numbers of young barracudas not more than 6 inches long and that "all lack the tooth of the lower jaw." The following data, however, may be given for specimens hardly more than one-third as long as Plée's. The four little barracudas taken at Tortugas in 1917, and described on page 59 for their color markings, have the great tooth of the lower jaw present and exposed for study by reason of the shrinking of the tissues at the tip of the upper jaw. The largest and bulkiest fish (2.6 inches long to the base of the caudal) has but one lower anterior tooth, the right. All three of the others (measuring 2.6, 2.35, and 2.25 inches) have but one each and that the left tooth.

After thinking on the matter for some time, it became clear to me that since the lower jaw is bilateral and since there is one fang at the external end of one ramus of the mandible, there is due to be one at the other. When the head of the fresh specimen from Miami was cleaned off, there (in the proper position), was the base of the broken-off left fang. A little dissection of the largest dried head revealed a precisely similar state of affairs. Removal of a lot of tissue at the apex of the lower jaw of dried specimen No. III showed a new tooth nearly ready to break through; and in the smallest dried head the stump of a broken-off tooth was found.

From a consideration of the foregoing facts it is clear that the great barracuda normally has two great teeth at the apex of the lower jaw, but for some unknown reason only one is commonly found, the one or the other being broken off. It would be of no small interest if the reason for this could be ascertained.

MANNER OF BREATHING.

In the preceding section, reference has several times been made and attention has been called to the figures of the breathing valves. These structures are developed to such a degree in the barracuda, that the fish is enabled to breathe with its mouth open or at most only partially closed. The head, including both the upper and lower jaws, is remarkably elongated and sharply pointed, as may be seen by reference to figures 3 and 4, plate II. Across the narrow anterior end of the roof of the upper jaw is stretched a fold of flexible membrane having a crescent-shaped posterior edge. In similar position in the floor of the mouth, just posterior to the symphysis of the mandibular bones, is a similar but larger breathing valve, whose hinder crescentic edge, however, swings free of the anterior end of the somewhat movable tongue. In this valve are marked depressions to receive the great premaxillary teeth. These valves may be plainly seen in figures 6, plate II; and 9 and 10, plate III.

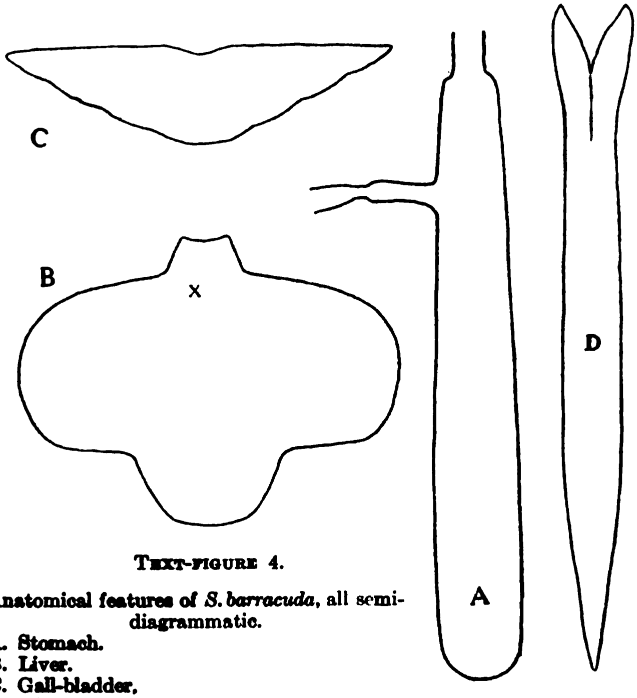
The action of these valves is very interesting. Ordinarily they lie horizontally, the one having above it and the other below it a space large enough for one to move about therein the handle of a scalpel, or, in a large specimen, one's finger. In gentle breathing, these valves swing slightly forward and downward for the one, forward and upward for the other. But in violent expiration, in consonance with the sharp upward rise of the floor of the mouth, the hyoid region, and the strong pulling together and downward of the gill-covers, the water would be forced forward out of the mouth but for these valves. Being elastic and markedly distensible, they swing together in a horizontal median line, catch and hold the forwardly moving column of water, which is then forced out backwardly over the gills and out under and behind the gill covers. With the relaxation of the hyoid region and opercula, the elasticity of the breathing valves brings them back to their normal horizontal position.

The reason why this fish has such well-developed breathing valves results from the fact that it does not close its mouth in expiration. This in turn is probably to be correlated with the extraordinary development of the great teeth, especially those on the anterior parts of the premaxillaries, since when the mouth is closed these must fit into the depressions in the anterior part of the mandibular valve. Because of these great teeth it is probably both more convenient and more comfortable for this fish to keep its mouth more or less open, even while breathing.

INTERNAL ORGANS.

Of no less interest than the structures just described are the internal organs. Of fish No. 10 (3 feet 10 inches in extreme length) careful dissection and full notes were made. The abdominal cavity was 18.5 inches long from the diaphragm to the anus, with a 1-inch post-anal extension to be described later.

There seems to be in this fish a structure apart from the pericardial sac which functions as a true diaphragm. Cuvier and Valenciennes (1829) speak of a diaphragm in the European form, the spet. Contained in the body-cavity were the spermaries, the air-bladder, and the alimentary tract with the liver and spleen. The digestive apparatus was 29.5 inches long, of which the stomach was 12.5 inches long by 1.5 inches wide, and the intestine 17 inches long by 0.5 inches wide. The stomach had the shape shown in text-figure 4 A, the intestine coming off 2.5 inches below or behind the œsophageal constriction. The sac below the pyloric opening was filled with the bones of fishes. My notes say that this organ was supplied with three large blood-vessels, but their connections were not worked out.



Text-figure 4.

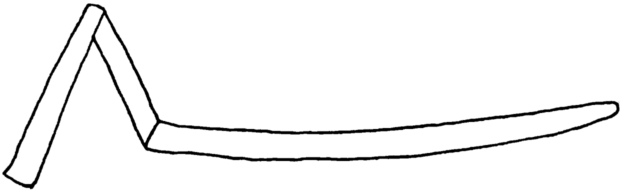
Anatomical features of *S. barracuda*, all semi-diagrammatic.

A. Stomach.
B. Liver.
C. Gall-bladder.
D. Air-bladder.

When the abdomen is opened the first organ visible in the anterior end is the liver. This is shaped very like a flat or English riding saddle turned upside down, the "skirts" or flaps extending upwards on each side of the stomach. It lies in the extreme forward end of the abdomen just behind the heart. In specimen No. 10 it was just about an inch thick at the forward end, where it was thickest (the point marked x in the diagram, text-figure 4 B). On the dorsal surface, opposite the point marked x, the bile duct, 3.5 inches long, was given off. This extended back to the gall bladder, which lay alongside the small intestine just anterior to the spleen. The gall bladder was 2 inches long and 0.5 inch wide, and was shaped somewhat like two conventionalized wings placed base to base, as shown in the diagrammatic figure 4c. The spleen had the same general shape, but was 3.25 inches long by 1 inch wide.

Lying between the body of the liver and the upturned "skirts" of the saddle (which were placed against the lateral walls of the abdomen) was the great mass of the pyloric cœca, which mass was shaped much like the liver, but had a greater volume and occupied a greater area. Of these cœca, only those lying on the dorsal and ventral surfaces of the mass were counted, but even these numbered 148. Dorsal to this mass of cœca were the stomach and small intestine.

The dorsal part of the abdominal cavity is occupied by the much-elongated air-bladder. Anteriorly this is bifurcated to form two horns, posteriorly there is but one which occupies the short post-anal extension of the abdomen (this was 1 inch long in a 3-foot 10-inch specimen). A fish 2 feet 10 inches in extreme length had an air-bladder 11.5 inches long, the anterior end of which had a 2.5-inch bifurcation. In a 3-foot 7-inch specimen, the air-bladder measured 15 inches, the horns being 3.5 inches in length. A third specimen, measuring 3 feet 10 inches between perpendiculars, had in its 18.5-inch abdominal cavity a 16.5-inch air-bladder, the bifurcation of which extended forward 3.5 inches. The general shape and appearance of this organ is shown in text-figure 4D.



TEXT-FIGURE 5. Spinal column of a fish found embedded in mesentery of a barracuda.

While dissecting this fish, some hard object was found embedded in the mesentery. At first it was thought that it was a mummified pipe-fish, but when freed of all attached material it was seen to be the backbone of a fish. This was confirmed when a portion of it was put under a microscope. It was fairly straight save at the upper end, where it was bent as is shown in text-figure 5. It is hard to conjecture just how this vertebral column could have worked its way out through the wall of the intestine into the mesentery. Inclusions, while not common, are not unknown. Some half dozen or more have been noted, but will not be considered here.

The first of the old writers to give any account of the internal organs of a *Sphyræna* is Rondelet (1558). His brief statement concerning the spet of the Mediterranean is as follows: "It has a long stomach with several additions [cœca], the bowels are long, the liver whitish."

Sloane (1707) briefly says that its stomach is "sack-fashioned," and that the cœcal appendages are very many.

Parra (1787) makes no mention of the viscera other than the air-bladder. This in his specimen was 12 inches long by 5 inches thick. His figure of the air-bladder, while crude, is in the main correct.

Cuvier and Valenciennes (1829) give a very definite description of the internal organs of a fish of the species described by Rondelet:

> "The viscera are simple, elongated. The liver has only one lobe, placed on the right side of the abdomen.
>
> "The œsophagus is short and soon is transformed into the stomach, which is an elongated but narrow sac of such a kind that the capacity of the viscera is not very great. The pylorus opens close to the cardiac end near to the diaphragm; it is provided with a very great number of cœca, disposed in a single row on the length of the duodenum. The intestine is narrow and goes straight to the anus without making a bend."

They note that the liver is black, rather thick, rounded at its extremities, and placed across the middle of the stomach. The swim bladder is large with the upper walls thicker than those next the viscera. It is pointed behind, but forked in front. Each horn is pointed and terminates near the skull, but no communication with the auditory capsules could be found.

Of our form, *Sphyræna barracuda*, Cuvier and Valenciennes merely say that its viscera are very similar to those of the European form, the chief difference being that its stomach is larger.

Where so many other structures were found to be so markedly individual it was something of a disappointment to find the reproductive organs entirely normal in structure and position. They were, however, very large, befitting a fish of this size. No. 10 (3 feet 10 inches over all) was a male with a spermary bifurcated in front but coalesced behind into a common tube ending in the genital pore. The right lobe was 8, the left 9.5 inches in length anterior to the point of bifurcation.

The gross structure of the ovary is precisely that of the spermary. My largest specimen (No. 12) was a female 4 feet 7 inches long over all. The right lobe of her ovary was 14.5 inches long, at the left sac 15.5—the eggs being immature. This huge ovary was presented to the United States National Museum, to the authorities of which I am indebted for the beautiful photograph which forms figure 14 of plate IV.

In the course of this research the only reference found to the structure of the reproductive organs is in Cuvier and Valenciennes (1829), and they merely note that the spermaries and ovaries are two straight sacs found in the hinder part of the abdomen.

HABITS.

Little is known about the habits of the barracuda, hence the few notes that have been collected in the course of this research may be of value. The fish seems in the main to be rather solitary. Only once was a school of them noticed. The water at Loggerhead Key being too shallow for an anchorage for our yacht, the *Anton Dohrn*, she was kept tied up to a buoy in the deep channel southeast of the sally-port of Fort Jefferson on Garden Key. Around the *Dohrn* thus anchored a small school of barracudas could sometimes be seen, and 6 or 8 fish were noticed on June 23, 1912. They were possibly attracted by the scraps thrown overboard from the galley; at any rate they were made bold by hunger, for, after one of them had been struck with the grains and wounded, another one of the school was taken on a trolling spoon thrown out and rapidly pulled in by hand. The appearance and attitudes of the members of this band are faithfully shown in Bullen's (1904) figure reproduced opposite page 55.

On one other occasion what may perhaps be described as a small school was seen. While trolling one day between Loggerhead and Bird Keys I had a heavy strike and, on hauling in, brought a large barracuda to the surface. Remarkable to say this had as a companion on either side a barracuda nearly as large as itself. These had their heads in the region of the right and left pectoral fins of the captive. For this no explanation can be offered. When the captive was brought nearer the boat, its companions disappeared.

The earliest reference to the fish called *Sphyræna* is in Aristotle's "History of Animals" (Book IX, chap. 3, 610*b*, 5), where it is listed among fishes that go in schools. Rondelet (1558), however, says nothing about such a habit, nor do Cuvier and Valenciennes (1829) refer to it, but they say of *S. guachancho* that "This species travels in companies, and there are sometimes taken together more than 200 individuals, all of the same size." Finally, Henderson (1916) speaks of the picudas or barracudas (maximum length 6 feet) ranging the water in schools or squadrons. Whether or not Cuvier and Valenciennes, and Henderson also, had in mind the subject of this paper, the great barracuda, can not, of course, be said. There are at least three species of the genus *Sphyræna* found in the West Indies, and all are commonly called barracudas.

In support of the present writer's contention that the big barracuda is solitary rather than social, Holder may be quoted (1903, p. 90; 1910, p. 125); and Holder has known this fish as no other scientific man ever has. This declaration of Holder's is concurred in by Bullen (1904), who says of the West Indian species that it is a morose and solitary fish, that even two are seldom seen together—in short that it seems to be a "comparatively scanty species." As to the California barracuda (*S. argentea*), Holder says that it "runs in schools, some of which have been seen miles in extent." And of the South Pacific form, Bullen (1904), speaking of New Zealand waters, says that "In no other place have I seen the Barracouta swim in schools of hundreds of thousands, almost as closely packed as mackerel." Ward (1907) succinctly says that on the east coast of Australia it goes "in schools which rival those of the herring and mackerel for numbers and denseness." At Beaufort, North Carolina, the present writer has frequently taken as many as a dozen of the young of *S. borealis* at one haul of a 100-foot seine, and it may be that the *young* of the big barracuda go in schools.

However, so far as the present writer's experience goes, the large fish are rather solitary, and such individuals are frequently to be found lying motionless near the surface of the water around large coral heads reaching nearly to the surface, around buoys, channel stakes, wharves, wrecks, etc. One or more individuals could be found almost every day of the season of 1912 "hanging around" our western dock at Loggerhead. Three other kinds of fish had the same habit at the same place, *i. e.*, gars, gray snappers, and "minnows." The last two kinds plainly came to get fish scraps from dissections and débris thrown overboard by the cook and they were prompt in their attendance. The gars, and in large degree the snappers also, fed largely on the minnows, while the barracudas fed on all three indiscriminately. Considerable sport was had shooting these gars with a 22-caliber rifle, but after wounding or killing them none was ever secured, for the barracudas snapped them up at once. However, in turn these were often taken with baited hooks.

The big barracuda has the interesting habit of herding its prey either until it has digested its previous meal and again feels hungry or because, being thoroughly savage and bloodthirsty, it enjoys the game. Perhaps both conjectures are correct. A number of instances of this sort have been noted. On June 9, 1912, off the southern coal shed at Fort Jefferson, a 30-inch barracuda was observed to have a small school of gray snappers herded in shallow water. Although I went quite near them, they paid little attention to me but huddled close together and as a flock moved one way or the other as the barracuda moved. They seemed to be in abject fear of it and made no effort to break away until I scared it off. In the same afternoon a 4-foot barracuda was observed under our eastern dock at Loggerhead herding a big school of fishes comprising some 150 gray snappers 12 to 16 inches long, and numbers of yellowtails, grunts, parrot-fishes, angel-fishes, surgeon-fishes, cock-eye pilots, etc. These hung around the piles and swam among the rocks piled on the foundations of the dock to strengthen it, and not one of them dared make a break for liberty. A trolling spoon was procured and thrown out to the big fish, whereupon he slowly backed off into deeper water, and *then* the assembly broke up.

One other instance of like kind may be given. During the first two weeks of June 1913, a big barracuda laid off our eastern wharf, herding the gray snappers and making it unsafe to bathe. Almost daily efforts were made to hook him, and a pair of grains was kept on that dock for his special benefit, but he avoided grains and eschewed hooks and herded snappers, until possibly he became careless. At any rate Capt. Wm. Lee Wilson, finding him engrossed with the snappers, broke his back with the grains late one afternoon and brought him in alive and kicking. This fish was a male 4 feet 1 inch long and weighed 21 pounds, the largest specimen but one ever taken by us. It is interesting to note that a barracuda thus engaged in standing guard over a herd of fish in the manner just described, will nearly always be found to have its broad forked tail slowly waving from side to side, vibrating very like the tail of a cat watching a rat hole. This has also been noticed by Holder (1908).

Of the breeding habits, absolutely nothing is known. Judging from the habits of the fish it seems probable that the eggs are pelagic. Thompson (1905) says that he took specimens 1.5 inches long inside the little sheltered lagoon of Bush Key. Still earlier, Holder (1903) says that at Tortugas spawning occurs in the spring, but adds that very young fishes are rarely seen, although specimens 8 inches and upwards are not uncommon. At Beaufort, North Carolina, the writer has frequently taken in July the 1.5 to 5-inch young of the northern barracuda (*S. borealis*).

No young barracudas were caught at Tortugas during the summers spent there by the writer, but 4 little ones were taken in the summer of 1916. Concerning these, Professor Longley (with whom I have had the pleasure of studying the fishes of Tortugas) kindly writes that on July 14 one about an inch long was dipped up somewhere out in the open. This "was marked with a distinct lateral band of brown pigment running the length of the body through the eye." The three others were taken on the west side of Bush Key; one about an inch long from over grassy bottom, the others 1 to 2 inches long from over sandy bottom. Dr. Longley thinks that all were probably swimming near the surface. These were unfortunately not preserved, since it was not known then that I was at work on this fish.

As noted previously, four young of the great barracuda were taken at Tortugas during the summer of 1917. They vary from 2.25 to 2.6 inches in length to the base of the caudal. The total length can not be given, since these specimens came to me dried and in handling their brittle caudal fins have lost their points. These are believed to be the smallest specimens ever studied.

Since writing the above, some corroborative data has come to hand and its inclusion here will be of value. Weber in his "Fische der Siboga-Expedition" (1913), in commenting on the widespread distribution of the Sphyrænidæ, says that this is probably due to the fact that the young stages are pelagic. He adds: "I captured in the sea far distant from the coast, in the surface plankton, a specimen only 13 mm. long of a Sphyræna species, which I have been unable to identify." He then adds that he has also taken four specimens of *S. jello* varying from 47 to 55 mm. long.

HOW THE BARRACUDA MAY BE TAKEN.

Taking advantage of the habit of the barracuda in basking near the surface of the water, and of its insatiable curiosity, it was our custom to have a trolling-spoon behind the *Velella* or the *Henderson* (our smaller launches) on their trips to and from the various collecting-grounds. In this way most of the barracudas studied were caught. These spoons were connected by a swivel to a "snood" of 6 or 8 feet of piano wire, and this in turn by means of another swivel was attached to the heavy cotton hand line. The wire was necessary to keep the fish from cutting the line, and the swivels to keep it from snarling the latter while endeavoring to break away.

Our largest barracuda was thus taken in the summer of 1913. In the latter part of the season we secured several good specimens, but during the first part the barracudas took our spoons in one, two, three order until it seemed that every big barracuda in the harbor was thus decorated. Chief engineer John Mills, however, provided some extra strong tackle, and with it was caught the largest specimen ever recorded from the Tortugas. This was No. 12 of the table, a female 55 inches long and 38 pounds in weight. Some of the incidents of its capture will illustrate the strength and vigor of these powerful fish.

While trolling one afternoon there came a tremendous strike which nearly pulled me overboard. Responding vigorously, this giant barracuda rose some 2 or 3 feet in the air.* The *Velella* was slowed down and I began pulling the fish in hand over hand as rapidly as possible. Captain Wilson jumped into the glass-bottomed boat, which was towing behind, and began helping me, the fish offering a vigorous resistance all the time, leaping and backing, and dashing from side to side. Finally, when the line had been hauled in fairly short, the fish ran under the skiff, caught Captain Wilson amidships with the line and slewing the boat around nearly threw him overboard; and even when safely slid over the rail into the boat, it threshed and hammered around at such a rate that it was feared that it would break the plate glass bottom; nor did it become quiet until it was soundly trounced on the head with a monkey wrench.

In the matter of the capture of the barracuda I am happy in being able to add further data from a man who can speak with authority, for Charles Frederick Holder lived as boy and man for many years on the outer Florida reef and knew it and its fishes as no other scientific man ever has. Among these fishes, he had much acquaintance with the barracuda. During several years spent at Fort Jefferson on Garden Key, he had great sport with the barracudas in the lagoon. He used the hand-line (trolled) sometimes, but being in search of sport rather than specimens, for the most part he trolled with a rod and light tackle. He generally had a white rag tied to a string 4 or 5 feet long trolling behind the boat, as a lure to "flush the fish." When the fish was "flushed" he would cast out his bait, which he notes must be a fish with bright shining silvery sides, and was rarely disappointed in getting a strike. Holder makes it plain that the fish falls a victim to its own inquisitiveness; but when a large specimen is hooked, and if the angler uses light tackle, there is a battle royal before the fish can be gaffed. He writes very interestingly (1903) of the barracudas in the lagoon at Tortugas.

---

*In none of the books and articles consulted in this study of the barracuda have any accounts been found of leaping by this fish save only in Büttikofer's book (1890). He says that on the coast of Liberia they often leap when in pursuit of smaller fish. My brother, while fishing among the Florida Keys, has had them leap when hooked.

The earliest writer to speak of taking them by trolling is Dampier, in his "Two Voyages to Campeachy" (1729, 6th ed.): "We commonly take them when we are under Sail, with a Hook towing after our Stern." Labat (1742) also notes that they are taken with the line and adds "by the use of the seine" also, but is careful to say that these latter are only small ones, not over 3 feet long. However, the first reference found to the use of a lure is in Macgillivray's "Voyage of the Rattlesnake" (1852). He says that in Bass's Strait (between Australia and Tasmania) they took them with "a hook towing astern baited with a piece of red or white rag." Once at Tortugas our stock of trolling spoons having been depleted, successful use was made of a small steel shark hook to which a piece of white canvas had been attached.

Bullen (1904) gives numerous instances of catching barracudas by trolling in various seas, and also tells of catching them by still fishing. He gives an interesting account of how the Maoris of New Zealand catch their barracudas by a kind of fishing which might be called trolling. This method, which is only possible because the fish go in great schools, is so absolutely novel that it seems worthy of incorporation here:

> "Take a stout rod, say 8 to 10 feet in length, or rather a pole, fairly rigid and tough, but not too heavy. To the end of it secure a piece of strong fishing line 5 or 6 feet in length. To the free end of this line attach a lure made as follows: A piece of red pine (*rimu*) 4 inches long, an inch wide, and half an inch thick, is scraped smooth and bright so that it will glow crimson when wet. Through one end of it is driven a 2-inch nail, which is carefully bent upward and filed sharp. Then this lure is fastened to the line in such a manner that, in case of the splitting of the wood, the fish shall not be lost. Now the boat, in which two fishermen sit to windward facing forward, is sailed briskly to and fro, the fishermen meanwhile whipping the water occasionally until a barracouta snaps at the bait and with a dexterous swing is flung into the boat, where, as there is no barb on the hook, he immediately

falls off. At the same time the peak halyards are let go, so that the boat's way is deadened and the fishermen ply their poles energetically. If they have struck a school, the fish rise and fall into the boat with rythmical regularity, every sweep of the bait into the water securing its fish."

The method, of attracting the fish by means of a lure and catching it by means of a spoon or a white rag on a hook, undoubtedly owes its success to the large inquisitiveness and utter fearlessness of the fish. To this desire to strike at anything flashing through the water, Bullen accredits the frequent loss of patent-log screw propellers, which are trailed behind vessels to give the rate of speed and register the distance traveled. This has been confirmed by Dr. A. G. Mayer in conversation with the present writer.

On rare occasions one of our laboratory men, all of whom were more or less expert with the grains, would "strike" a specimen. Thompson (1905) says this may be easily done; but we found the fish too wary for much success along this line unless its attention was distracted, as was the case of specimen No. 11 previously described.

Holder in another book (1908) gives an interesting account of how a boatman of his named "Barracuda" used to take the fish of the same name with the grains. Trolling a white rag behind his rowboat as a lure, he would scull the boat into the sun. In this way the fish would face the sun and be dazzled by it, while the striker would have his back to the luminary with everything behind the boat clearly illumined. When the fish was thus brought within range of the grains, the boat would be suddenly stopped, and as the fish, still watching the lure, would forge slightly ahead on one side, the grains would be thrown.

FOOD AND FEEDING.

This fish is as strictly carnivorous as the shark, although hardly so indiscriminate in its choice of flesh. So far as my observations and dissections go, it is wholly a piscivore, feeding entirely upon other fishes. It is not meant to convey the idea that it will refuse other flesh food, but that left to itself its staple food is fish. In this I am happily corroborated by Linton (1910), who found in the stomachs of ten Tortugas specimens, collected in the summers of 1906, 1907, 1908, no other food than fishes.

With regard to the food of the big barracuda and the condition in which it is taken in, dissection of fish No. 12 (55 inches long) gave valuable data. Its stomach was enormously distended and, when opened, was found to contain some 5 pounds of fish in large fragments, surrounded with a lot of smaller fragments and topped off with the latter half of a fair-sized Margate grunt in a rather advanced stage of decomposition. The fish merely chops up its prey and swallows the large fragments whole.

Holder (1903) found that the barracuda could be taken only with a bait of shining-sided fish, and that it scorned all other baits, including the much-vaunted and everywhere successfully used "crawfish." Probably the curiosity of the fish is attracted by the silver-sided fish as it is by a trolling white rag or spoon There is no evidence whatever that it at any time eats "crawfish," as the large spiny crustacean (*Palinurus*) of the reef is locally called.

Most writers on this fish say that it is carnivorous, but most of them are so obsessed with its apparent desire for human flesh that they refer to no other source of food. However, Cuvier and Valenciennes (1829) speak of a spet of the "middle sea" having its stomach filled with atherinas and little clupeids. Bullen (1904) dissected specimens from the Indian Ocean and found their stomachs filled with small mackerel. The man-eating habit referred to will be discussed in another section.

With regard to its piscivorous feeding habits, Bullen (1904) tells a story which is worthy of condensation and reproduction here. It seems that while the oceanic waters around New Zealand abound in fine food-fish, the fresh waters are almost totally devoid of fishes worth taking for either food or sport. Consequently, at great expense of money, time, and trouble, salmon eggs were sent out many years ago and planted in one of the rivers. Some of the eggs hatched, and some of the young survived. These grew apace and finally reached the stage when, following their natural instincts, they journeyed seaward. Down in the estuary of their river they first tasted the salt water, but here some native barracudas were prowling around in a school seeking what they might devour, and few if any of the young salmon ever went back to their place of birth. This story illustrates not merely the feeding habits and voracity of the barracuda, but the necessity of knowing the natural history of the native animals of a country before attempting acclimatization of new ones.

PARASITES.

From what has been said as to the food and manner of feeding of the big barracuda, it is to be expected that it would be the host of all sorts of entozoa, parasitic helminth worms, but strange to say such is not the case. Having myself paid no attention to such parasites in my specimens, I have naturally turned to the writings of Professor Edwin Linton. Fortunately Professor Linton has spent considerable time at Tortugas studying its parasitic entozoa, and among the fishes examined were numerous specimens of *S. barracuda*. In 1908 he notes that 3 large and 4 small barracudas were examined, and in these immature nematodes were found for the most part encysted in the viscera. Likewise a few trematodes were found. So few were parasites of all kinds that Linton notes:

> "It is perhaps worthy of remark that the great barracuda, which is a very voracious and predatory fish, appears to harbor but few parasites, either as a final or intermediate host. This conclusion is warranted also from the

results of the examination of five barracuda in Bermuda in 1903. The largest Tortugas specimen measured about 1.5 meters in length; the Bermuda specimens were about half that length. It would be of interest to know whether the apparent immunity from parasites of the barracuda and other fish is correlated in any way with the digestive ferments."

In his later paper dealing with the trematodes, Linton (1910) notes the examination of eight barracudas, in every one of which he found specimens of a new or at any rate undetermined species of *Gasterostomum*. This seems to have been the only parasitic trematode which he found in the barracuda.

However, an *Ascaris* has been found by Linstow (1906) in *Sphyranura barracuda* from Tasmania. Linstow's generic name is presumably a misspelling of *Sphyræna*. Other than the references given, no accounts of internal parasites of the barracuda have come to light. The fish seems singularly free from such unwelcome guests.

We now turn to an animal which in the past has been considered as an ectoparasite to its hosts, the barracuda included. I have among my notes gravely worded accounts of the finding, when the so-called parasites had been removed, of places worn in the skin or scales of the host; further it has been accused of living on the blood of its host thus obtained through the skin; and, most preposterous of all, one account specifically states that when one had been removed from the bottom of the boat to which it had adhered, the planking was found to be injured. Reference is made to the sucking-fish, *Echeneis naucrates*, for small forms of which the great barracuda sometimes acts as host.

On July 4, 1914, while trolling east of Loggerhead Key, the writer took a barracuda 40.8 inches long. When hauled in it was very active and called for strong repressive measures before it was quieted. When the *mêlée* was over there was found clinging to the deck the smallest example of *Echeneis* I had ever seen. This fish was about 4 inches long and had a most remarkable tail, plumose instead of crescent-shaped. It was carried to the laboratory and, as it seemed sick and the hour was late in the afternoon, it was put into an aquarium to be studied the next morning. When morning came it was gone and no trace of its whereabouts or its manner of going was ever found. Some two years later I was greatly interested to read in the "Memorias" of the Cuban ichthyologist, Poey (1856-58), the following description of a little *Echeneis* which he calls "*E. sphyrænarum*, the sucker of the Picudas."

"This little fish has never been found up to the present time save only on the *Sphyræna picuda*. It hides itself among its hosts' gills and escapes therefrom when the large fish is taken. The individual which I describe is 75 mm. long. In size [depth?] it scarcely exceeds a centimeter, since it is shrunken by concentrated alcohol and its body is greatly diminished. One sees, however, that its structures are all elongated. The eye is contained 5 times in the length of the head. The disk ends towards the [anterior] third of the pectorals, and has on each side 10 lamellæ furnished with spines in one rank alternately long and short. The mouth is homodont: the teeth of the jaws and vomer are cardiform, but the lower jaw shows on the outside a peculiar character; it projects, is a little enlarged, ends squarely, and bears on each side toward the point an external row of five hooks (crochets), strong, and pointed, remarkable for their development. The pectorals arise near the opercles, and between their points and the origin of the vertical fins the distance is equal to the length of the head. The unpaired fins are opposite each other and have the ordinary form. They arise at an equal distance from the point of the snout and the end of the tail, the point not being considered. This point, formed of the two middle rays, is 1½ times as long as the rest of the caudal, a character very distinctive of this species. The dried condition of my specimen forbids a count of the rays. The color is a very dark blue verging on black. The pectorals are white except at their bases. The two anterior points of the vertical fins are white, as are also the upper and lower edges of the caudal fin, but not the point which terminates it."

One other reference may be cited just here: Lütken, writing in 1878, speaks of an *Echeneis lineata*, a fish identical with Poey's *E. sphyrænarum*, which he found among the collections in the museum of Copenhagen. This was taken in the South Atlantic from a *Sphyræna barracuda*. Lütken thinks that this particular Echeneis is to be found attached only to the sphyræna.

Mr. Peter B. Roberts, keeper of the fish market at Key West, Florida, has collected for me a considerable number of small specimens of *Echeneis*, ranging from about 4 to 7 or 8 inches long. I wrote him asking from what fish these were taken, and his answer was "that the sucker-fish are found on almost every fish in the waters around here, but the greater numbers are found on what are commonly called around here the amber-jack and the black grouper—that is, the smaller ones like you wanted collected."

I then wrote him that a certain small sucker-fish was thought to be found only on the big barracuda and asked for information on this particular point. Now, Mr. Roberts, by virtue of his position at Key West and his long experience both as fisherman and distributor of fishes, has an exceedingly wide and accurate knowledge of the fishes of that region. He writes:

"In answer to your questions I would like to state that the fishermen say that there is only one kind of [striped] sucker-fish, and that the small ones are not a distinct kind from the larger ones, but as they grow they naturally cling and stick to the larger fish. The ones found on the barracuda are the same as those found on other fish, the larger they grow the larger the fish they go after. . . . I would like to say . . . that I think that they are all the same kind, both big and small, simply growing through the different stages of life."

After the receipt of this letter the little suckers sent me by Mr. Roberts were carefully examined; 3 were found to have 19 lamellæ, 9 to have 20, 8 had 21, 6 were supplied with 22, 5 with 23, 1 with 24, and

2 had 25. The smallest had pointed tails, *i. e.*, had the central caudal rays longest and all had tails more or less plumose like that of the lost specimen above referred to. Presumably some of these were taken from barracudas. According to Jordan and Evermann they fall into the species *E. naucrates* (XXII to XXVIII lamellæ), or to *E. naucratoides* (XX to XXI lamellæ). At any rate, none of these can be identical with Poey's *E. sphyrænarum*, which had only 10 lamellæ. Gill (1862), in his criticism of Poey's identifications, calls this fish *Phthierichthys lineatus*, the striped louse-fish. Jordan and Evermann (1898) follow Gill, and note that this little sucker-fish is found attached to barracudas. This, however, is probably an echo of Poey. At any rate, the point as to whether or not the big barracuda is the sole host of this particular sucker-fish is worthy of further investigation.

USE OF THE BARRACUDA AS FOOD AND POISONING RESULTING THEREFROM.

There is a long-standing prejudice against the use of *Sphyræna* as food. Salviani, the Roman physician and student of fishes, says in his book published at Rome in 1554, that "At Rome they are justly held to be common or cheap fish, nor do they have any proper fashion or mode of rendering them savory." Cuvier and Valenciennes, in referring to Salviani's statement, say that other writers, presumably contemporary, accord it a comparison with the haddock, everywhere esteemed as food; and that still others say that its flesh is light, friable, and of good flavor. Rondelet (1558) likewise testifies that its flesh is white and pleasant to the taste.

With regard to the use of the West Indian barracuda as food, there is likewise a widespread and long-standing prejudice based on centuries-old allegations of its poisonous qualities. This is such an interesting and important point that it will be taken up in detail and an effort made to get at the truth and its explanation. This belief, so far as the writer knows, was first noted by Du Tertre. He writes as early as 1667 that the flesh is like that of the pike, but dangerous to eat since it is sometimes poisonous. He then tells us that to determine whether it is hurtful it is necessary to examine the teeth and liver. If the former are white and the liver sweet-tasting, it may be eaten with impunity; but if the teeth are black or the liver bitter or harsh, it "ought no more be eaten than arsenic." As an explanation of the origin of the poison, he says that in the West Indies in his day it was thought to be due to the fact that the fish eats the fruits of the very poisonous manchineel tree which have fallen in the water. That this explanation has persisted we will see later. And since this explanation of the poisonous quality of the flesh of the barracuda is repeatedly offered, the following interesting corroboratory note seems worth giving.

Dampier (1729) in his first voyage to Campeachy landed on the Isle of Pines on the south side of Cuba near the west end. Among the animals of which he makes mention are large land crabs. Of their feeding he says:

> "The Manchaniel Fruit, which neither Bird nor Beast will taste, is greedily devoured by them, without doing them any harm. Yet these very crabs that feed on Manchaniel, are venomous both to Man and Beast that feeds on them, though the others are very good Meat."

There is now to be quoted an account which, because no certain fish is named, may seem of doubtful relevancy, but which, as the sequel will show, is of direct value to the matter in hand, and in all probability relates to the very fish under consideration. In the Philosophical Transactions of the Royal Society for 1675 there is published an extract from a letter of one Mr. "J. L." to the publisher concerning poisonous fish in the Bahamas. It reads as follows:

> "The Fish that are here, are many of them poysonous, bringing a great pain in their joynts who eat them, which continues for some short time, and at last with two or three days itching the pain is rubbed off. Those of the same species, size, shapes, colour, and taste are one of them poyson, the other not in the least hurtful. And those that are, are so only to *some* of the company. The distemper to Men never, that we hear of, proves mortal. Doggs and Cats sometimes eat their last. In men who have once had the disease, upon the first eating of the fish, though it be those that are wholesome, the poisonous ferment in their body is revived thereby, and their pain increased."

There is another account, nearly 200 years later, from the pen of the English surgeon, Morton (1868). Commenting on the great variety of fish caught at Nassau, he adds:

> "Some of these fish, at certain times of the year, are very unwholesome, and, when eaten, give rise to severe purgings, vomiting, and cramps. During our stay, four men belonging to a coasting vessel were poisoned, one of whom died from eating part of a large barracouta, which they had caught. This fish, when large, is said to be very unsafe food, and great risk is run in eating it. The one which gave rise to fatal results in this instance, was upwards of five feet in length."

Sir Hans Sloane (1707), in volume II of his "Natural History of Jamaica," says of the barracuda:

> "According to its feeding on venemous or non-venemous Food, 'tis wholesome or poysonous to those who eat it; 'tis also noxious in some Seasons of the Year, and in some Places, and innocent in others, I suppose according to its Nourishment, by which now and then, it acquires so much poison as to kill immediately."

However, Dr. Patrick Browne (1756) says, of the two species which he found in Jamaican waters, that "they are both firm and palatable fishes, much esteemed by many people."

Dampier (1729), in his "Second Voyage to Campeachy," thus speaks of the edibility of the barracuda:

> "They are firm well-tasted Fish; but 'tis dangerous eating them, for some Men have been poisoned with them. Divers Persons are of the Opinion that these Creatures are poysonous in some Places only, and that but at some Times of the Year. I know that in many parts of the West-Indies, some have been injured by eating them, and that at different Seasons of the Year; therefore Seamen commonly taste the Liver before they venture any further; and if that has a biting Taste like Pepper, they esteem the Fish unwholesome, but if not they eat it: and yet I found even this Rule to fail too. I judge the Head and the Parts near it, to be chiefly venemous."

Labat (1742) discourses at length upon the edibility of the barracuda, whose flesh he says is white, firm, rather oily, and almost of the same taste as that of the pike, but at times is poisonous. He offers a very interesting explanation:

> "As it is extremely voracious, it eats greedily everything which it finds within and on the water, it happens very often that it encounters *Galeres* [sea-nettles, medusæ, "Portuguese Men of War"] or the fruit of the manchineel, both of which are very violent and caustic poisons. The Becune does not die because it has eaten them, but its flesh contracts the poison and causes death in those who eat it just as if they had eaten the dangerous fruit or the *Galeres*."

To tell whether the flesh is good or dangerous, Labat would inspect the teeth. If they are black it is dangerous; if some are white and some black, then taste the liver. If it is bitter, reject the fish.

Catesby (1754) writing of the Bahama barracuda gives some details not found in any earlier writer. He evidently wrote of large specimens, for he says:

> "The flesh has a very rank and disagreeable favour [flavour?] both to the nose and palate, and is frequently poisonous, causing great sickness, vomiting, and intolerable pains in the head, with loss of hair and nails; yet the hungry Bahamians frequently repast on their unwholesome carcasses."

Fermin (1769) says that the *becune* has firm, white flesh, somewhat oily, but of very good taste. However, it is not to be eaten save after taking the precautions noted above. He explains the poisonous properties of its flesh by its voracious feeding habits, especially by its feeding on the manchineel.

In 1808 a Dr. Chisholm published an article on the poison of fish. His observations were made while a resident of the island of Grenada, where he appears to have practiced medicine. He knew of and probably treated cases of poisoning resulting from eating the barracuda. He says that the fishermen account for this poison by alleging that the fish are poisonous only at the spawning season when they repair to and feed upon "sea-moss" [which Chisholm identifies as *Corallina opuntia*]. This poisons them and they in turn poison those who eat it. After their return from the banks, the poison gradually leaves them and they become wholesome again.

Chisholm notes that copper is supposed to be the essential basis of this poison, but adds that he knows of "no facts which decidedly prove this." As to the value of salt as a preventive he says:

> "A barracuda, the poisonous quality of which was proved by its entrails killing a cat which had ate of them, being cut into slices or junks, and slightly salted or corned, was rendered perfectly wholesome, and, as usual delicious to the taste. Instances have occurred, however, in which salt has not exhibited its counteracting power."

Moreau de Jonnes, as early as 1819, and 1821, made extensive and intensive studies of poisoning resulting from eating fishes, the barracuda among others. In his later and fuller paper, he quotes various authors that crabs which eat the fruit of the manchineel become poisonous (thus confirming that very accurate observer, William Dampier), while those which have no access to this fruit are wholesome. He then takes up and disposes of the alleged causes of the poisonous quality of the flesh of certain fishes. He states that the first cause commonly assigned is that the fishes eat poisonous zoophytes. This he rejects because poisonous fish are found where these are absent and wholesome fish where they abound, because the same fish where these abound are not poisonous throughout the year, and lastly because he fed poisonous hydroids of all kinds to fishes and then fed these fishes to various animals and to man with impunity. The second reason adduced is that the fishes are poisoned by copper. Moreau de Jonnes, however, notes that copper is present and fishes wholesome on the English coast and that copper is absent and fishes poisonous in the West Indies, and finally that oysters grow on the copper bottoms of vessels and have been found wholesome when eaten. Then he takes up the manchineel theory. He notes that no one has ever proved that fishes eat the manchineel fruit, and that he has made many dissections of poisonous fishes (barracudas among them) without ever having found fragments of the manchineel fruit in them. He thinks that the eaters would be killed by the eating, and that, since these trees abound widely on seashores, there should be many more poisonous fish and crabs than there are. Finally, for himself he concludes that poisoning from eating fishes (barracudas included) is due to the fact that the flesh has some inherent poisonous properties, or develops such morbid qualities as a result of the hot climate. While our author gives the former explanation his very strong belief, we have in the second a premonition of the ptomaine theory.

Another physician, William Ferguson, writing some years later (1823), declares that the size of the fish has nothing to do with its poisonous qualities, nor does the use of salt destroy its noxious properties. Then he refers to a report from a physician in Martinique concerning a family which had been poisoned by eating a barracuda which had lain in salt 24 hours.

The most extensive and possibly the most interesting account of poisoning by the barracuda is given by Cuvier and Valenciennes (1829) in their treatment of *Sphyræna barracuda.* The M. Plée quoted seems to have been a French naturalist who lived in the West Indies. Apparently his manuscript was sent directly to Cuvier, who writes in the first person:

> "All that has been reported concerning poisonous fishes of warm countries and that malady called Siguatera, which is found under certain circumstances, has the power of inspiring curiosity and interest, so that I have thought I ought to insert here the data collected by M. Plée on the barracuda, just as I have found it among the papers of that unfortunate naturalist.
>
> "'Some persons,' said he, 'fear to eat this fish, because it has been frequently proved that it is the cause of illness and sometimes of death. This poisonous property of the becune is present very certainly in a particular state of the individual fish, which appears to show itself at different seasons of the year.
>
> "'I have consulted several persons with regard to the poison of the becune and all have assured me that there is an infallible means of satisfying one's self when one comes from fishing for it whether or not it is poisonous. He has only to note in cutting it whether or not there runs away a kind of whitish water, or rather a kind of *sanie* [*i. e.*, serum or exudation], which in all cases is a sure sign that the becune is in the diseased condition of which I have spoken above. D. Arthur O'Neill, Marquis del Norte, has told me that he has often made experiments on dogs and that all these have confirmed the sureness of this means of safety.
>
> "'The signs of poisoning by the becune are a general trembling, nausea, vomiting, sharp pains, particularly in the joints of the arms and hands. Sometimes these symptoms follow each other so rapidly that it becomes extremely difficult to determine in a precise fashion the different stages of this deadly affection.
>
> "'If death does not put an end to this malady, as happily is most ordinarily the case. one may sometimes see how the virus always causes certain singular pathological phenomena. The nails of the hands and feet gradually die and drop off; the hairs, which, as is well known, are of the same nature as the nails, finally drop out also. These phenomena have been noted in several individuals to have continued for a considerable number of years. One case may be cited in which this experience persisted for more than twenty-five years.
>
> "'One remarkable fact is that when the becune has been salted it never causes any trouble. At St. Croix for instance, it is the custom not to eat it until the day following the one on which it was salted. May it not be that the salt is an antidote for the poison of the becune?'"

However, M. Plée adds in honesty that which spoils an apparently straightforward piece of testimony by saying that he has never seen a case of barracuda poisoning, but has had his information from persons "well instructed and worthy of confidence."

Widespread is the belief that this reported poisonous affection of the flesh is due to the fish's feeding on substances containing copper, as referred to in Chisholm's account. Gosse (1851) refers to the same thing when he says that "the colonists believe [that it] is owing to its feeding on submerged 'copper-banks.'"

Poey (1856–58) writes that its flesh is excellent, but is forbidden to be sold in the markets because it is sometimes dangerous, and gravely affects the health of those who eat it at such times. The poisoning caused by fish is called *Ciguatera* and the best antidote for it is the juice of the citron. He then adds that such poisonings are rare but serious when they do happen, especially when caused by eating the picuda. Last of all he naively concludes that "One may eat it with full security if one will in advance try it on a cat." He adds that it is the belief of the fishermen that the fish is unsuitable for food if the roots of its teeth are blackish, but that he has no first-hand knowledge of the matter.

Duméril (1867) has gone very thoroughly into the matter of poisoning from eating fishes. He quotes a Dr. Court, a practitioner on the island of Trinidad, that the smaller barracuda species is harmless, as are small individuals of any fish held to be poisonous. The great sphyræna is often very poisonous. In addition to the teeth-liver tests, a new one is here given:

> "When a silver spoon or coin, placed in the vessel in which the fish is being cooked, does not become blackened, the flesh may be eaten without fear."

Duméril takes no stock in the copper-bed theory, but does suggest another cause for the poisoning. A Dr. Guyon, "sanitary inspector of troops at Martinique," communicated to Duméril some considerable data and: "According to him the real cause is a commencement of decay in the flesh of the fish" [accompanied by a giving off of H_2S as shown by the silver coin test of the preceding paragraph]; and Duméril adds: "This opinion I also share." Here we have the first definite statement for our fish of what later has come to be known as the ptomaine theory of fish poisoning.

The prejudice against this fish seems to exist to-day throughout the West Indies. It is, however, a good food-fish. In our mess at the station we frequently ate *S. barracuda*. The small forms 18 to 20 inches or thereabouts, were excellent. Fish No. 3 of the table (page 58), 30.5 inches long, was pronounced by the mess to have a good flavor, somewhat like that of a flounder. Larger and older fish are likely to have coarse flesh, rather oily, and a somewhat characteristic odor. Such were used only for shark bait.

Temminck and Schlegel (1850) say that *Sphyræna obtusata* was fished for in the bay of Nagasaki, Japan, where it was regarded as a delicious food by the Japanese. While for *S. guachancho*, Guichenot (1853) quotes Ramon de la Sagra that its flesh is savory and excellent for the table, and unlike that of the becuna, is never poisonous and hence may be eaten without danger of Ciguatera. Day (1865) adds similar testimony for the Indian form, *S. jello*, when he says that it is sometimes eaten by Europeans though not particularly esteemed.

Büttikofer (1890) says the same for this fish in Liberia.

In his "Introduction to the study of fishes" (1880), Gunther notes that barracudas are commonly used as food, but sometimes (particularly in the West Indies) their flesh manifests poisonous qualities due to their having preyed on poisonous fishes; but as to the South Seas form (probably *S. commersonii*) he apparently quotes Andrew Garrett (1877), that it is freely eaten and highly valued as a food-fish.

Saville-Kent (1893), writing of this same form, affirms that in eastern Australia it is much esteemed as a food-fish. Macgillivray (1852) bears similar testimony for the Australian barracuda.

Bullen (1904) says that he has eaten the barracuda in the West Indies, around New Zealand, at the Cape of Good Hope and elsewhere, and has always found it a palatable food-fish. It seems to be especially prized for this purpose in Australia and New Zealand. In this, Ward (1907) is in hearty agreement. The California species, it may be noted in passing, is also excellent for the table.

In this connection Jordan (1905, II, p. 223) notes that the flesh of larger forms is often difficult of digestion and when eaten frequently results in serious illness. Of smaller specimens "The flesh is firm, delicate, and excellent in flavor." In the first volume of the same work (A guide to the study of fishes), Jordan discusses in very illuminating fashion the various forms of poisoning due to eating fishes. Quoting from Dr. Jacques Pellegrin, he says:

> "The flesh of fishes soon undergoes decomposition in hot climates. The consumption of decayed fish may produce serious disorders, usually with symptoms of diarrhœa or eruptions of the skin. There is in this case no specific poison, but the formation of leucomaines [ptomaines?] through the influence of bacteria. . . . It is especially severe in certain very oily fishes, as . . . [the barracuda]. The flesh of these and other fishes occasionally produces similar disorders through mere indigestion. In this case the flesh undergoes decay in the stomach."

I have not been able to find Pellegrin's paper (Les Poissons Vénéneux, Paris, 1899), but the abstract of it in "Revue Scientifique" for the following year contains for the barracudas the mere statement that the poisonous qualities of the flesh of these fish have an origin very difficult to establish.

In a recent bulletin of the New York Zoological Society (Nov. 1916) Mr. L. L. Mowbray examines with some care the matter of fish poisoning, which he calls "Ichthyotoxismus." Mr. Mowbray is an experienced collector of fishes for the New York Aquarium and has operated for many years in the Bermudas, Bahamas, and Florida Keys, particularly around Key West. He has studied at some length in all three localities the question of poisoning following the eating of fishes, particularly the barracuda, and has come to the conclusion that it is simply ptomaine poisoning. Mr. Mowbray's data are so definite, so much to the point, and tally in so many points with the reports quoted above, that it will help clear up the matter to quote him in full. He writes:

"While in the Turk's Islands I questioned many fishermen concerning the fishes that were poisonous, the effects of the poison, and at what seasons the fish were most dangerous. Without exception their reports tallied. All agreed that there were two forms of the disease; that the fish from the north side of the Islands were the most dangerous, those from the south side not being so likely to prove poisonous. This seems incredible, as the island of Grand Turk, most densely populated of this group of islands, is only 1.5 miles wide by 6 miles long, and lies in the trade winds and the Bahama Current, which move all surface food at a considerable rate to the westward. I consulted Dr. Geogaghan, then the medical officer of the colony, who kindly gave me a description of the symptoms, which he had personally experienced in both forms of the disease. Dr. Geogaghan said:

"'To my knowledge the common poisonous fish are barracuda, jack, and muletto kingfish. In certain places, for some reason or other, the barracuda is more likely to be poisonous than if caught elsewhere. There are two distinct kinds of poisoning from these fishes. The ordinary type is similar to ptomaine, being in the nature of a simple gastro-enteritis of an irritative sort. It is characterized by acute spasmodic pain in the stomach, diarrhea, and vomiting, coming on from 10 to 20 hours after eating the fish, and subsiding readily under treatment. There is occasionally headache, usually fever (101° to 102° F.) and a rapid pulse (90 to 100). Generally speaking, it is an acute gastro-enteritis.

" 'The other form is in the nature of a toxemia. I have never seen a case following on the eating of jack, but can not be certain on this point. The symptoms are slow to subside, sometimes lasting for months. It starts from 2 to 6 days after eating the fish, very seldom less than 2, and usually 3 or 4. There is repeated pain of a dull resistant type over the region of the pancreas; constipation; slow aching pains in the joints, especially in the knees and back, without any physical signs; pain behind the eyes and headache, acute irritation of the bladder with frequent burning and tickling sensation.

" 'The joint pains are called "bone-pains" here, and are similar to the pains of influenza, though more particularly associated with the joints. There is an intense feeling of lassitude and debility, and subnormal temperature.

" 'Naturally clinical cases vary in severity. Occasionally the two forms of poisoning are combined, one following the other. I look on the first as a simple irritative disturbance of the intestine which throws off the irritant in the usual way. The second is a real poisoning of the system. I have had both myself and it was many months before I was rid of the joint-pains of the second.'

"The Turk Island species described herein are also among the principal food-fishes of Key West and the Bermudas, excepting the kingfish, which is seldom taken at Bermuda, and poisoning is unknown in these localities.

"After observing the conditions and the manner in which the fish are handled, I have reached the conclusion that the reason they are poisonous in one region and not in another, is that in Bermuda and Key West almost all fishing boats have live-wells, and therefore usually bring their fish to market alive, while in the Turk Islands and Bahamas the fish are killed and allowed to remain in the sun until the shore is reached—sometimes 5 or 6 hours after they are caught.

"All of the fishes considered poisonous are of soft flesh and rich in gastric juices, and are therefore the most likely to decay quickly; and, when eaten in a partially decayed condition cause *ptomaine poisoning.* Naturally some

> are more poisonous than others. Those caught in the mornings are exposed to the sun's rays much longer, and are therefore much more decomposed.
>
> "The fishes, when examined externally and internally, appeared to be in the finest condition when caught, and I could detect no difference between them and those of Bermuda or Key West. I have seen specimens at Grand Turk Island with the scales standing almost on edge through the decomposing of the flesh, which, forming gases, expanded the fish. These fish are frequently sold from house to house, though caught the day before and in a half-putrid condition. It is probable that if, when caught, the fish were eviscerated and bled, a case of poisoning would be a rarity.
>
> "An interesting antidote for fish poisoning is used by the natives. When a fish has been eaten that is suspected to have been poisonous, the bones are saved for 24 hours, within which time, if at all, symptoms should appear. On the first indication of trouble, the bones are roasted, pulverized, and made into tea for the patient. Belief in the efficacy of this treatment is implicit, provided the bones of the right fish have been used."

In order to get at present-day notions at Key West, I again called on Mr. Peter Roberts for information and he very kindly wrote me as follows:

> "As to the barracudas, it is not a certain time of the year that they are poisonous, but it is those of a certain kind that are poisonous. The only barracudas eaten around here are the ones caught along the shore and in shallow water. The barracudas that are poisonous are the ones that are caught in the Gulf Stream, and the people around here are very careful not to eat any of them. They are of a distinct kind, known as the 'Blue Backs,' and can be readily distinguished from the non-poisonous kind."

In this connection it is of interest to note that the beautiful colored figure of *Sphyræna barracuda* in Cuvier and Valenciennes (reproduced herein as figure 18, plate v) (1829) is yellow on the sides below the lateral line and on the fins and tail, while the back is a beautiful *blue*, the blue of the deep blue sky, almost as blue as deep sea-water. Furthermore my notes record that specimen No. 10 of the table, on page 58, had a blue sheen on the dorsum in certain lights.

Captain W. L. Wilson, previously referred to as one of our boatmen at Tortugas, was born and reared in the Bahamas. I wrote to him at Nassau about this matter and he replied:

> "The old and large barracudas are, as a rule, poisonous. I have never known or heard of one under 3 feet being poisonous. After the fish are cooked you can tell if they are poisonous by taking a piece of the cooked fish and breaking it apart. If you find very small dark veins the fish is all right, but if there are no dark veins found it is poisonous."

The latest information on barracuda poisoning is from the pen of Stephen Haweis (1917) in his book on the sea gardens of Nassau, Bahamas. It seems to be solidly a quotation from the distinguished English ichthyologist, C. Tate Regan, the original of which I have been unable to locate. This statement is as follows.

> "The largest of these species (*Sphyræna barracuda* or *picuda*) is a valuable food-fish, but it has long been known that individual specimens may be poisonous, causing severe illness and even death. There is evidently something more in this than ptomaine poisoning due to eating fish that is not fresh and the probability is that the poisonous quality of the flesh is not directly caused by the barracuda feeding in certain places or on smaller poisonous fish. It seems more likely that barracuda are poisonous when suffering from an infectious disease. Plée has stated that when barracuda are poisonous this can be recognized by a thin white fluid running out of the flesh when it is cut, whilst Poey says that the poisonous barracuda have the teeth blackish at the roots. However, no real investigation of the matter on scientific lines has been made, and it is quite likely that were such investigation made, the cause might prove to be quite different from what has been supposed."

None of the encyclopedias, not even the eleventh edition of the Britannica, contains the word "ciguatera." Parra (1787) has a short chapter bearing the heading "Ciguatera." This has been carefully translated for me, but nowhere does it contain any reference to the barracuda. Parra gives at some length and in very clear and minute detail an instance of ciguatera poisoning of himself and his family. This agrees in general with the accounts given above. He refers to the manchineel theory, but thinks it of no value since in certain parts of the West Indies where these trees abound the disease is wholly absent. He confesses himself entirely in the dark as to the cause and also the best treatment, lemon juice being the thing which affords some and possibly the most relief.

We have here some exceedingly interesting accounts of barracuda poisoning and some equally interesting if divergent theories in explanation. The present writer having had no personal experience or observation of it can not express any personal opinion. The flesh of the larger fish is coarse and oily and has neither appetizing appearance nor odor. Such flesh might well be provocative of gastric disturbances if eaten. With us at Tortugas such was used only for shark bait. Again it is quite possible that certain species might be poisonous on account of their feeding habits, and an even more plausible supposition is that these fish may be poisonous at certain seasons only of the year. As for the first it is well known that certain species in a group are poisonous while others are not. This finds explanation in the fact that certain poisonous alkaloids are found concentrated in some organ. These are most apt to be found in the ovary, and are most abundant and dangerous at the breeding season.

In the second place, it might well be that the barracuda, being a piscivore, might feed at certain seasons of the year on fishes which at that time were poisonous, and thus itself become poisonous. This might also lead to its being poisonous in certain localities only.

However, whatever other causes may give rise to barracuda poisoning, there can be no doubt that decomposition products, ptomaines, formed by the action of saprophytic bacteria, must be reckoned with. These bacteria, as Fischel (1892) states, might be present in the flesh or blood, as such or as spores, or might get into the flesh through wounds and bruises, or by the intake of infected food. However this may be, fish (especially such as have oily flesh like the barracuda) "go bad" quickly under the tropical sun and when eaten in such condition would give rise to ptomaine poisoning.

SIZE.

The big barracuda justifies its colloquial name by growing to great size. My largest specimen was 4 feet 7 inches long and weighed 38 pounds. This is the largest specimen recorded from the lagoon at Tortugas. However, one day I saw, under the wharf-house at Garden Key, a specimen that looked to be fully 5 feet long. As the *Velella* pulled out, I threw my "spoon" overboard, knowing that the fish would probably follow the boat, and hoping to get a strike. My hopes were realized, for before we had gone a hundred yards I had a strike which in 10 seconds left me with only a hundred feet of line and a groove burned in my right fore-finger just the size and depth of the line. The fish, with the spoon and remainder of the line, was not seen again.

Vincent, in his interesting, "Sea fish of Trinidad" (1910) speaks of somewhat similar experiences with blistered hands while trolling for what he calls the "pirate of the seas." But this is not unexpected when we read that his largest specimen measured 8.5 feet.

At Miami, Florida, on visiting a local taxidermist's shop, I found its walls almost lined with the mounted skins of big fellows 4 to 6 feet in length. These were taken on the Florida Reef some 6 or 8 miles away. Jordan and Evermann assign 6 feet as the average maximum size for the West Indian specimens, and this is corroborated by Henderson (1912), while Poey (1856) states that the Havana fishermen say that it sometimes attains a weight of "fifty livres" which would indicate a length of over 6 feet. As noted above, Holder has had wide experience with these fish and he states (1903) that he has taken them in the Florida Keys between 6 and 7 feet long and weighing 60 to 70 pounds. However, he adds that he has heard of specimens even larger than these.

One of the older writers on the natural history of the Antilles, Rochefort (1665), gives the length of the barracuda as from 6 to 8 feet with a girth in proportion. Du Tertre (1667) quotes Rochefort with approval, but gives no figures of his own. Sloane (1707) had only small specimens, but indicates that it grew to a large size. Labat (1742), however, makes up for any deficiencies by declaring that "They have been seen in this river [Gallion] 18 to 20 feet long and of the size of a horse." His statement, however, must be taken *cum grano salis*. Catesby (1754), speaking of Bahama fish, is more moderate, though his figures come close to Labat's: "This fish grows to a large size; some of them I have seen 10 feet in length, and some I was told are much larger; though the more common length is that of about 6 or 8 feet." However, for present-day Bahama fish, Captain Wilson, who has seen hundreds, writes me that the largest measured 6.5 feet. But, Fermin (1769), writing a few years after Catesby, says that along the coasts of Surinam they were occasionally taken approximately 15 feet in length. These were certainly giants.

A photograph was made of the 55-inch specimen (38 pounds), but the photographer in developing the plate unfortunately broke it. Figure 1, plate I, is a photograph of a medium specimen which, however, contrasts well in size with the 11-year-old boy standing beside it. These fish, however, were small compared to the one figured by Wood-Jones. (See figure 2, plate I, of this paper.) Saville-Kent (1893) does not portray the Australian form, but notes that it sometimes attains a weight of 50 pounds, from which one may judge that such would be between 6 and 7 feet long. Günther (1877) quotes Andrew Garrett that the Polynesian form (probably *S. commersonii*) grows to 8 feet in length and 40 pounds in weight. This must be a very slender fish, as is the California *S. argentea* (maximum length 5 feet). However, the form found on the west coast of Africa grows larger. Büttikofer measured a *S. jello*, caught in the mouth of Cape Mount River, Liberia, which was 10 feet long.

However much the authorities may vary in their estimates of the size of the barracuda, all agree that that size is *great*, though it does not have the bulk of the jewfish nor that of some of the great groupers, and when along with its size consideration is given to its remarkable swiftness and its implacable temper, it must be acknowledged (the sharks alone possibly excepted) as the real ruler of the Gulf-Caribbean waters.

THE BARRACUDA DANGEROUS TO MAN.

All the various recitals above will lead the reader to ask if this fish is not dangerous to man. The answer is that in southern Florida it is more feared than the shark. If the reader will now turn to figures 7, 8, 9, and 10, plate III, showing the teeth, and to pages 62 and 63, on which the formidable dental armature of this fish is described, he will find abundant reason for the dread generally had for this ferocious fish. But he needs to be informed of the utter fearlessness of the fish, of its ferocity, and of its insatiable curiosity.

The ordinary shark is usually an arrant coward. If a shark is "hanging around" a boat or wharf, and a man falls overboard or any large object is thrown overboard, generally that shark will depart in a panic. One day, when we were cruising around off the municipal slaughter-house at Key West, where there were some half dozen 10-

foot tiger sharks (*Galeocerdo tigrinus*) swimming around just out of harpooning distance, I questioned one of the most experienced of Key West fishermen (then in my employ) as to the danger of falling overboard in such a locality. He replied that there was practically none, and when, to draw him out, I professed to disbelieve him he coolly offered to prove the matter by jumping overboard *on* one of the sharks if I would steer him up close enough. So confident was I of the accuracy of his statements that, had the sharks not been wanted for specimens, I would have taken him at his word. Similarly the paddling and splashing of a number of swimmers will ordinarily drive off any shark. It must, however, be borne in mind that I am speaking of my experience and observations at Beaufort, North Carolina, and in southern Florida only. For an incident illustrative of this character of shark at Beaufort, see the account given by the present writer in 1912. But it is not thus with the big barracuda. He is inquisitive, utterly fearless, and seemingly of implacable temper. The Key West fishermen dread this fish much more than they do the ordinary shark. The whole matter was admirably put 250 years ago by the Sieur de Rochefort (1665) in his "Natural history of the Antilles":

> "Among the monsters greedy and desirous of human flesh, which are found on the coasts of the islands, the Becune is one of the most formidable. It is a fish which has the figure of a pike, and which grows to six or eight feet in length and has a girth in proportion. When it has perceived its prey, it launches itself in fury, like a blood-thirsty dog, at the men whom it has perceived in the water. Furthermore it is able to carry away a part of that which it has been able to catch, and its teeth have so much venom that its smallest bite becomes mortal if one does not have recourse at that very instant to some powerful remedy in order to abate and turn aside the force of this poison."

One of the points above noted is expressly corroborated by Fermin (1769), who says in so many words that with its long trenchant teeth it is able to cut clear through and carry off anything which it encounters in swimming—a statement which may well be believed.

The other points in Rochefort's accounts are confirmed by Du Tertre (1667), who tells us that:

> "This fish [which grows to a length of 8 feet] is greedy, blood-thirsty, bold, and is more dangerous than the Requiem [shark] . . . , because besides the fact that it can bite more easily, it is not startled by any noise any more than by the movements which may be made in the water. On the other hand, in order to investigate these, it launches itself at the persons [making these movements] in order to devour them."

Sir Hans Sloane (1707) gives some particulars of especial interest when taken in connection with other accounts to follow later:

> "It is very voracious, and feeds on Blacks, Dogs, and Horses, rather than on White men, when they can come at them in the water."

This is concurred in by that keen observer, William Dampier. In his "Two voyages to Campeachy," which appeared in sixth edition in 1729, he says of "Parricootas," which he describes as long fish, having round bodies, and long mouths with sharp teeth, that:

> "They commonly haunt in Lagunes among Islands, or in the Sea near the Shore. They are a floating Fish, and greedily take the Hook, and will snap at Men too in the Water."

To Pere Labat (1742), of the 18 or 20-foot barracudas, we owe some interesting data and even more interesting conjectures. On the question of its danger to man he writes:

> "As it is not obliged to turn on its side like the shark when it wishes to bite, it is infinitely more dangerous. Our savages, who attack and kill Requins [sharks] and Pantoufliers [hammerhead sharks] with knives, do not dare to run that risk with Becunes, because, moving with such extraordinary speed, they carry away an arm, a leg, or a head as if they had been cut off with a blow of a sabre. It has happened several times that horses and other animals crossing [the river Gallion] by swimming have had their legs cut off or half their bellies carried away."

Going further into the matter, he writes:

> "One is assured by many experiences that voracious fishes like the Requin, the Pantouflier or Zygæna, and the Becune attack more often a dog or a horse rather than a man, and a negro rather than a white man, when by reason of the overturning of a boat or canoe they find these different species of animals in the sea. I leave it to the curious to seek the reason, it suffices that that which I report is a veritable fact and testified to by those who have accurate knowledge of America and of other regions where these carnivorous fishes are found. My notion is that the bodies of dogs and horses give off 'corpuscles' which strike the fishes more strongly and attract them longer. Just as we see that wolves, crows, and even dogs more often come to carrion or to a body in which corruption has begun, rather than to a body which has recently been deprived of life. To my mind also not only are 'corpuscles' exhaled by them in large quantities but also they extend farther and strike more strongly the organs of those animals."

This recalls and substantiates what Sloane has written on the same subject (see page 95). But Labat further says:

> "But a thing rather surprising, yet one which is however of public notoriety, is that these same fish more often attack an Englishman than a Frenchman, when they find them both together in the water. It may be that the Englishman has pores more open than the Frenchmen, and as a necessary consequence he will exhale more corpuscles proper to strike the organs of these fishes and hence attract them."

Labat next goes on to argue at some length that there is a difference in the "corpuscles" given off by members of two nations because of a difference in their foods and in their physical habit of body: that the Englishman, being a heavy eater of meats and of a hearty rugged habit of body ("beefy"), all in marked contrast with the more delicatebodied and daintier-feeding Frenchman, will "produce an exhalation of corpuscles whose odor is more penetrating, which scatter farther, and which strike more on the organs of these animals." This conclusion he backs up by quoting the cannibal Caribs that the flesh of an Englishman is more appetizing than that of a Frenchman. He also declares that the Carib trackers can follow an Englishman or a negro through the forests more easily than a Frenchman by simply smelling of their tracks, and by the odor can distinguish the nation of the track-maker. Then he argues that if this is so for men, why should it not hold also for the fishes.

Just what to make of this the present writer does not know. The whole matter depends on the strength of the sense of smell in fishes. This, in sharks at any rate, is quite acute. By pouring blood or by hanging overboard a carcass (the more decomposed the better) sharks may be readily enticed up current or up tide. This the present writer has done (1912). The odor from the negro is very offensive to the white man, and I have been told that to the Chinaman the odor of the white man is just as distasteful. As Labat says at the close of his discourse, each must be left free to make up his own mind as to the value of this conjecture.

Catesby's remarks (1754) on the danger to bathers of this fish are brief but to the point. "It is a swift-swimming and very voracious fish, preying on most others; and some of the largest size have frequently attacked and devoured men as they were washing in the sea." While Brown (1756), without specifically saying so, indicates his knowledge of similar habits on the part of the Jamaican fish.

Bullen (1904) quotes the apocryphal stories current throughout the West Indies as to the diabolical ferocity of the barracuda and ends by giving an eye-witness account of the fear of this fish which is universal throughout these islands. A pair of can-hooks had been lost overboard in 40 feet of water, and for a small reward a band of 8 negroes, swimming about the vessel and paying no attention to some sharks in the near vicinity, endeavored to recover these. All went well until the cry of "couter," "couter," was raised, whereupon bedlam broke loose. Crazed with fear, the negroes fairly climbed over each other to come aboard by the help of ropes flung out to them. Bullen adds that even when safe on the ship "their demoralized, panic-stricken condition was painful to witness." If the reader will, in this connection, examine Bullen's well-drawn figures of the barracuda (reproduced herein opposite page 55) and the other figures given in this paper of the head and jaws, and will recall the cold ferocity of this fish, he can better understand the truth of this story.

Holder (1908) had in his employ many years ago as guide and factotum on the outer Florida Reef a typical reefer who went by the common name "Barracuda." Because he was an expert at taking this fish with the grains, Holder thought that he had been so named, but inquiries showed a totally different origin. It seems that years before this man had lived on Sea Horse Key in the Bahamas and had there got his name because of a horrible experience he had had with the barracuda fish. On the occasion of a great storm, a small ship was driven on the reef and all on board washed overboard and drowned save one woman who was lashed to the rigging. This man, McNally by name, threw off his clothes, tied a light line around his waist, and despite the dissuasions of his friends began his hard swim to the vessel. Several times on his journey he was seen to strike at something, and on his return (having sent the women in tied to the line), he was seen to fight with something and once was pulled under. His friends thought that he had encountered a shark, but when he came to shore it was found that both going and coming he had been attacked and seriously bitten by the fish whose name he afterward bore.

Further, Holder was told of a number of men who had gone overboard in channels between the Keys and who had been almost killed by these vicious fish. He also relates an incident, which seemingly fell under his own observation, of a barracuda which had been forgotten in the well of a fishing sloop. When a man went down into the well to repair it he was attacked by the fish and maimed for life.

Finally, as bearing out the present writer's statements at the beginning of this section, Henderson (1916) may be quoted that:

> "As to the *picona* danger, not much can be said beyond mentioning the general fear of this aggressive fish. . . . He prefers rocky places about the reefs, where, lying motionless near the bottom, he darts at his prey with a swiftness that baffles the eye. His sinister appearance, astonishing quickness, and occasional habit of ranging the water in schools, like squadrons of submarine destroyers, have combined to give him a bad name. . . . As a matter of fact we feared these [barracudas] more than sharks."

Captain Wilson writes of the Bahama barracuda:

> "I know of a case of a man who was walking quickly on a shallow reef beside deep water, and a barracuda flashed up and hit him on the foot. In this case, the cause, I think, was his white foot going in and out of the water quickly, for anything that moves quickly they will flash at. They give a ghastly bite."

Thus we have abundant evidence of the ferocity of *S. barracuda* in Gulf-Caribbean waters and of its dangers to man. Turning to the Pacific, it is interesting to note that the California form, *S. argentea*, which attains a length of 5 feet, is entirely harmless. In the southern part of this great ocean, however, is found the formidable *S. commersonii*, which has been known to reach the great size of 8 feet. On the authority of Andrew Garrett, Günther states that such large individuals are extremely dangerous to bathers. Much more circumstantial, however, is the following interesting present-day account from Wood-Jones (1912). In this connection see his figure of such a giant fish reproduced herein as figure 2, plate I. He writes:

> "The barracouta has, however, left its mark upon at least one man, and I have seen a Cocos [Keeling Archipelago] native the whole of whose calf muscles had been torn away by the cruel teeth of this large [marine fish] relative of the pike. The barracouta is an ugly and dangerous fish, for it is of all sea creatures the most difficult to see, and it has a habit of dashing upon whatever stirs in the water."

In his very interesting book, describing the cruise of the *Curaçoa* among the South Sea Islands in 1865, Brenchley figures and describes the tie-beam of a house at Uji, Solomon Islands. On one side of this beam is carved and painted a scene showing an overturned canoe with the men fighting with sharks and other fishes which are devouring some of them. Concerning this scene Brenchley says: "Among the fish regaling themselves on the remains of the bodies which they have partially devoured are to be found more than one species. The long central fish is the Sphyræna, popularly known as the Barracuda." This tie-beam was brought away by the *Curaçoa* and figures of its two sides form the frontispiece of Brenchley's book. The figure of the barracuda, with a piece of human flesh in its jaws, is admirably drawn and perfectly recognizable.

Further west in the Indian Ocean the barracuda abounds in the waters around the island of Mauritius, where it bears the local name *tazarre*. Nicholas Pike, while United States consul at Port Louis nearly 50 years ago, made many valuable natural history observations. From his delightful book "Sub-tropical rambles" (1873) we learn that, whenever he went collecting in the tide-pools and over the shallow reefs, he always carried a fish spear to protect himself against attacks from barracudas and eels. He tells us that once he was attacked by a *tazarre* of considerable size, which "came right at me like a bulldog." He harpooned it in the side, but it got away and then came at him the second time. This time he struck it in the head, but held it off with difficulty, though he was a large powerful man.

HABITAT.

The barracudas comprise the sole genus of the family Sphyrænidæ. There are some 20 species of these carnivorous salt-water "pikes" found in the warm seas (tropical and sub-tropical) all over the world. Jordan and Evermann (1896) list 7 species from North American waters: 2 from the Pacific Coast; 4 from the Atlantic; and 1, a European form, from the Bermudas only (on the authority of Dr. Goode). Of the Atlantic forms 3 seem to be wholly tropical, ranging from Pensacola and Charleston to Bahia; 2 of these are occasionally taken as far north as Wood's Hole, probably as stragglers in the Gulf Stream; the other (the northern barracuda), seems to range from Cape Cod south to about Cape Fear. The young are not infrequently taken in Beaufort Harbor, though the adult is unknown there. So far as its habitat is concerned, the northern barracuda seems to be a decidedly aberrant form.

Equally aberrant is the form found on the west coast of Africa. Reeve reports (1912) that it goes up the Gambia River, 160 miles, to McCarthy Island, where the water is perfectly fresh. He notes that such specimens are very thin, and conjectures that they find it difficult to get sufficient food. Whether this barracuda is the *Sphyræna jello* reported by Büttikofer for the Liberian coast, as elsewhere noted, can not be said.

FOSSIL FORMS.

Our knowledge of the Sphyrænidæ far antedates the historic period; even Aristotle's mention of them is comparatively recent. In the Cretaceous seas which covered Kansas and the adjacent parts of the Great Plains region, and in the bays and shallow sounds along the North Carolina coast, there disported themselves in large numbers certain fishes of powerful frame and voracious habits to which the paleontologists have given the name *Protosphyrænidæ*, the first sphyrænas. The generic name was assigned to these fishes by Leidy as early as 1857, but it seems that Agassiz much earlier than this had examined the teeth of the same form and had erroneously placed their owners among the Saurocephali. There is, it must be confessed, some doubt about the affinities of these fishes. Indeed, Jordan (1905) says "the jaws are armed with very strong teeth, as in the barracuda, which however the species do not resemble in other respects."

Two of these Protosphyræna fish are known by the specific names of *Protosphyræna nitida* and *perniciosa*. Careful perusal of the literature would probably give the names of a number of other species referable to this fossil genus. However, it is not the purpose of the present writer to go into the matter of fossil forms further than to call to the attention of the reader the fact that the fossil Protosphyrænids are by many considered as the ancestors of the modern Sphyrænids. So Felix (1890) seemed to think, and his figure of *Protosphyræna nitida*, reproduced herein as figure 25, plate VII, certainly does show the head of a fish whose teeth and jaws are remarkably like those of the big barracuda. Attention is called to the two great canines in front and to the knife-shaped teeth of the upper and lower jaws. The teeth of the upper jaw are seen outside those of the lower, but whether this is natural or an artifact can not be said—possibly both, and rather certainly the latter, since the great canines are also found outside the lower jaw-bone. However, from the drawing they seem to be rooted in the premaxillary.

Louis Agassiz (1843) also figures in the atlas to volume V of his great work on fossil fishes two skeletons of certain fishes which he does not hesitate to call *Sphyræna*. These skeletons are from the Eocene of Monte Bolce in Italy. The first is called *S. bolcensis*, from its place of discovery, and is based on a number of skeletons from different museums. The second is *S. gracilis* and several specimens are known. These are reproduced herein as figures 23 and 24, plate VII. Attention is called to the shape of the snout and to the upper teeth in figure 24, and to the 24 vertebræ (the proper number) in figure 23.

In 1901, the distinguished paleontologist Dr. A. S. Woodward catalogued the Sphyrænid material in the British Museum and reduced most of the previously described forms to synonymy. He sets up three distinct species: *Sphyræna bolcensis*, *intermedia*, and *suessi*. Six other species are quoted from earlier writers, but Woodward thinks the material from which they were described to be so imperfect (in one case consisting of a single tooth only) as not to justify the erection of species upon it. All the valid material is from the Eocene of Monte Bolce near Verona in northern Italy. Woodward throws out Agassiz's *S. amici* from Mount Lebanon as not belonging to this genus.

NOMENCLATURE.

The generic term *Sphyræna* means hammer, according to Jordan and Evermann (1896), and hence the barracuda is a hammer-fish—a total misnomer since it resembles nothing so little as a hammer, as was noted so long ago as 1554 by Salviani. This erroneous derivation and interpretation seem to have come about somewhat as follows: The name *Sphyræna* originated with Aristotle, who, in his "Natural History," book IX, chapter 3, 610*b*, 5, simply names the fish. However, it will be shown further on that the more common name among the Greeks was *cestra* (a kind of light javelin invented and used during the Persian war) and also a goad or pointed stick. The word *Sphyræna* apparently has its origin in the word *sphyra*, a hammer, but *sphyræna* has no use or meaning other than as the name of the fish under consideration. This statement is based on the authority of Professors Gildersleeve and Miller of the Johns Hopkins University. The name *cestra*, however, seems to be a definite allusion to the shape of the fish and its pointed head.

Our next authority is Pliny. In his "Natural History," book XXXII, chapter 11, paragraph 54, he speaks of the fish "called *sudis* in Latin, and in Greek *sphyræna*, names which indicate the shape of its snout." This *sudis* or *sudes* was a kind of javelin and also a kind of stake somewhat pointed and hardened in the fire. Furthermore the colloquial name used along the Mediterranean shores of Italy, France, and Spain to-day is spet or spetto. This term has already been used in this paper in speaking of the European fish, and refers to the elongated form and pointed snout.

The etymology of this name is very obscure. *Sphyræna* = hammerfish is such a misnomer that I set to work to puzzle it out. Rondelet (1558) makes *sphyræna* = *cestra*, a sharpened stake, because of its pointed snout. Cuvier and Valenciennes (1829) cast strong doubt on the hammer-fish derivation and definitely state their belief that *cestra* (javelin or stake) is a synonym for *sphyræna*, in allusion to its pointed snout. This led me back to Conrad Gesner's "Historia Animalium," IIII, where was found a wealth of material which is summarized below. In this connection I wish to express my thanks to Professor C. W. E. Miller, of the Johns Hopkins University, to whom I am indebted for translations of and some keenly critical comments upon the names used. These translations and comments have gone far towards clearing up the situation and for confirming the authors referred to in this paragraph.

Before dealing with the data found in Gesner, it may be well to state that Aldrovandi (1613) is in full accord with Gesner, whom he quotes in large degree.

Gesner (1558) quotes the Greek poet and philosopher, Epicharmus, who flourished at Syracuse about 485 B. C., where he speaks of "*cestras* and shining perches." Next he notes that Speusippus (about 407 B. C. to 339 B. C.), the nephew and disciple of Plato, likened the *sphyræna* or *cestra* to the fish called in Latin *acus* (gar-pike). Then he finds that Athenæus, the Greek philosopher of Naucrates and Alexandria, Egypt, in his great work, "Deipnosophistæ" (about 200 A. D.), writes that "Dorian says that what they call the *cestra* is the *sphyræna*, and when Epicharmus called it the *cestra* he no longer said *sphyræna*, although they are the same. And the Attic Greeks more often call the *sphyræna* the *cestra* and very seldom use the name *sphyræna*." These quotations make it clear that the two names were used interchangeably but that *cestra* gradually came to be used almost exclusively for the fish once called *sphyræna*.

Thus *sphyræna*, *cestra* (or *kestra*), and *sudis* were all used synonymously for a certain slender sharp-headed fish recalling the idea of a javelin, a pointed stake, or the prow of a ship. Now there enters on the scene one Theodorus Gaza, a learned Greek scholar who was born about 1400 and who died about 79 years later. The Turks having captured his birth-place, Thessalonica in Macedonia, he was driven into Italy, where he was for many years professor of Greek at Ferrara. Later he moved to Rome and engaged in translating Greek works into Latin, among them Aristotle's "Natural history of animals" (1476). Gaza translated *sphyræna* = *kestra* = *sudis* by the Latin word *malleolus*, hammer, and hammerfish it has remained to this day.

Now *the* hammerfish is *Sphyrna zygæna*, the hammerhead shark, and on this point Gesner definitely says that the common codices or texts of his day were full of errors, *zygæna* being often written for *sphyræna* and *vice versa.* The hammerhead shark gets its name (*sphyrna,* hammer; *zygæna,* yoke) because the laterally elongated lobes of its head stand out from the body, giving the head end of the fish the shape of the Greek letter *τ.* Further, Gesner says that at Marseilles there was a hammer-fish called Jew-fish, which by many was thought to be a *sphyræna* but which was really a *zygæna.* Then he quotes the French ichthyologist Gilles, that the hammer-fish of Marseilles was a *zygæna,* and that it was called a Jew-fish because its lateral head projections much resembled a kind of head-dress having lateral horns formerly worn by certain Jews of that city. Furthermore, it is interesting to note that the specific name given to the hammerhead shark by many ichthyologists is or was *malleus* (hammer). From all this we may easily see how the names were confused.

However, this etymological tangle is not so easily unraveled as the preceding paragraph seems to show. *Sphyræna* probably has its origin in the word *sphyra,* which means hammer. Gaza gives as a synonym the Latin word *malleolus,* and this has added further to the complexity. The word *malleolus* means a little hammer, and Professor Miller writes me that it is neo-Latin for the tibia and fibula with their enlarged ends. These in Greek are represented by the word *sphyron,* which also means hammer. And so it seems that we have come to a *cul de sac.* Now let us return to *cestra* and see what we can get from it.

Pollex (born at Naucrates, Egypt, about 130 A. D.) has left us a dictionary of Greek words in which "*cestra*" is defined as "a certain kind of hammer"; but what kind? Professor Gildersleeve writes that it was "an agricultural implement employed in breaking up clods." And Professor Miller says that it was a double-headed hammer, flat on one head and with the other pointed, a pick hammer or "*Spitzhammer,*" such as geologists use. Such hammers are figured among the illustrations in archeological works. Now the error of preceding writers is clear. Deriving *sphyræna* from *sphyra,* hammer, and either overlooking *cestra* in its meaning of javelin, or using *cestra* as hammer without going into critical study of what kind of hammer, they have in all cases made *Sphyræna* the hammerfish, when it should be the *pickhammer* fish, the name being given not in allusion to the hammer end of the tool but to the pick end. Hence *Sphyræna* is not the hammerfish but the *pickhammer* fish, and it is seen that the name plainly alludes to the shape of the head and snout, and that it is a synonym for *cestra* and for *sudis.*

On the origin of the specific name, barracuda, I regret that I am unable to throw any light. Walbaum in 1792 seems to have first used it for the name of the species. So struck was he with the similarity of this fish to the fresh-water pike, that he named it *Esox barracuda.* He, however, simply gave his so-called "pike" the native name barracuda with which Catesby had labeled his drawing from a Bahama specimen in 1731.

The use of the name, however, antedates Catesby. Sloane (II, 1725) uses it. Dampier, whose sixth edition (published 1729) I have, refers to this fish under the name "Parricoota." Dampier's second visit to Campeachy, where he first saw or at any rate described the "Parricoota," was made in 1676. In book II, chapter 2 (p. 144), in which the fish is referred to, Dampier says that "About the middle of February 75–6, we sailed from Jamaica" for Campeachy and after a short and safe voyage arrived there. Dampier's Voyages are written directly from his manuscript journal, and the accounts on pages 171–2 of his printed book are dated 1676, so at least to this date the name *Parricoota*, a corruption of the apparently native word *barracuda*, can be traced. Probably a close search of the early Spanish chroniclers and writers on the natural history of the West Indies would show the name in use long before Dampier's time.

This name, in its various spellings (barracuda, barracouta, barracuta, parricoota, paracuta, etc.), has become wide-spread, being the common name for the fish wherever found the world around.

The other colloquial name, picuda, seems to have been given the fish by Parra in 1787. This is a Spanish term, having its root in common with our English word pike, given in plain allusion to the similarity in form and habits between this fish and the fresh-water pike.

The name becune is French in origin, and it will be recalled as the name used by Rochefort, De Tertre, Labat, and Fermin. This is the Gallicized form of the medieval Latin word *becuna* according to the Century and Standard Dictionaries. However, not being content with this, I asked Professor Miller to pass on these names also, and he kindly writes that *bécune* is French and is borrowed from the Spanish *bécuna*. He notes that the first syllable of these words corresponds to the French word *bec*, beak of a bird or snout of a fish, and that the Latin word *beccus* is of Gallic origin. The *-une* or *-una* is simply a termination. Hence *becune* or *becuna* means *beak-fish*.

In this connection, Professor Miller makes the interesting suggestion that, since *sphyra* conveys no suggestion of sharpness or pointedness, as does the word *cestra*, possibly the name may have been given in allusion to the hammer-like swiftness and force of the fish's attack. Then he adds: "The *sphyræna* would then be hammer-fish; the *cestra*, the pickhammer-fish; the *sudis*, the stake fish; the *becuna* (*becune*), the beak-fish: according to the varying point of view of the observer."

DRAWINGS OR FIGURES OF THE BARRACUDA.

The history of the big barracuda has been rather fully given in the course of this paper, and little can be added here. However, it may not be without interest to give some few points about figures of the sphyræna, both European and American.

Belon seems to have published the first known figure of the European sphyræna, but Cuvier and Valenciennes say that it was incorrectly drawn. I have not seen this, nor have I had opportunity to examine Rondelet's original figure published in the Latin edition of his book in 1554. His figure in the French translation of the above work is the earliest examined in the course of this research, but there is no reason to doubt that it is not identical with that in the 1554 folio. The figure is small and not very distinctive. Cuvier and Valenciennes say that the head is too long. This figure was copied by Gesner in the fourth volume of his "Historia Animalium" (1558). Aldrovandi (1613), however, had a new drawing made for his book, but our French critics say that it was made from a dried specimen. While in some ways an improvement on the preceding figures, it is not a good drawing, the head especially being poor. These writers also say that Bloch's figure —which I have not examined—is the best made up to that time, but that it is faulty in some respects.

However, of the figures studied, Salviani's (1554) is far and away the best portrayal of the sphyræna, either European or American, made prior to the time of publication of Cuvier and Valenciennes' elegant drawing to be referred to later. Occupying a whole page in Salviani's folio, it is well drawn and well printed. There are some defects. The teeth are not figured absolutely correctly and the ventral and caudal fins are not well done, but the figure as a whole is distinctly good. The eye is fine, the head generally well done, the lower jaw is longer and has a distinct lump at the upturned end. The point of the upper jaw is plainly upturned and slightly hollowed out for the reception of the great tooth at the point of the lower jaw. The fins are well placed, as is also the lateral line. Most noticeable is the absence of the first dorsal fin and this absence is severely criticized by Cuvier and Valenciennes. However, Salviani distinctly says that there are two dorsal fins, "the first on its middle, the hinder one, however, towards the caudal." In the dead fish, this is always sunk in the sheath, and so it was in the specimen from which Salviani's artist made his drawing. It is greatly to be regretted that this striking figure was not studied until the present paper was in press. Had it been seen earlier it would have been reproduced herein.

The earliest figure of the American fish which has come to light in the course of this research is Rochefort's drawing of the *becune* taken from the edition of his book published in 1665. It is a poor figure, giving only in very general outline the form of the fish and the relative position of the fins. The most striking defect consists in the absence of one set of the paired fins, just which it is hard to say. However, historically it is of enough interest to be reproduced herein as figure 15, plate v. There is reason to think that this figure appeared in the first edition of Rochefort's work in 1558, which I have not been able to consult.

The next ancient figure is that in volume II of Sloane's "Voyage to Jamaica" (1725), table 247, figure 3. This, however, is so poorly drawn as to have nothing about it to distinguish it as a barracuda save the name appended below. For these reasons it is best to omit any reproduction of it here. Almost as bad is Labat's (1742) reproduction of Rochefort's figure (1667), since Labat leaves off the lateral line, possibly because Rochefort had drawn it incorrectly.

Next in point of time comes Catesby's drawing of the Bahama form. While this is crudely done it seems worth while from the historical standpoint to reproduce it in this paper as figure 16, plate v.

The next figure of the big West Indian barracuda is Parra's drawing, reproduced herein as figure 17, plate v. It was published in 1787 and, though crude, is by far the best of all the figures published prior to 1829.

The first really accurate delineation of *Sphyræna barracuda* is that found in the third volume of Cuvier and Valenciennes. This is really a wonderful drawing to have been made from a preserved specimen. The reader will find it given herein as figure 18, plate v. The one criticism is that the head is somewhat too short and blunt.

After Cuvier and Valenciennes' elegant figure, the next portrayal known to me is that found in volume IV of Jordan and Evermann's great work on American fishes. This excellent figure has but two defects worthy of serious criticism; the lateral line runs straight where it should rise over the pectoral fin; and the fin rays do not correspond to the count in the text. This drawing is herein reproduced as text-figure 2. These same authors, in their "American food and game fishes" (1905), publish an excellent figure, apparently a photograph of a preserved, possibly a still fresh specimen, viewed from the side and slightly from below. This fine figure shows the lateral line markedly arched over the pectoral fin, but the authors persist in their statement that it is straight.

Very valuable are the three portrayals in the plate taken from Bullen's book (1904) and reproduced herein facing page 55. These are by long odds the best figures extant of this fish, and portray very closely attitudes in which the fish has often been observed at Tortugas.

The observations on the living fish, its structures and habits, and the collection of material and data for further study were made at the Tortugas Laboratory of the Carnegie Institution of Washington. Here Dr. A. G. Mayer, the director, did everything possible to forward my work. The historical side of the paper was worked up in the Library of Congress and the Library of the United States National Museum. To the officials in charge of these two great libraries I am under obligation for many courtesies. The extracts from the various authors referred to are in the main literal quotations. Transliterations would possibly have enhanced the appearance of the paper, but experience has taught me that verbatim quotations are far more valuable to the reader.

LITERATURE CITED.

Agassiz, Louis. 1843. Recherches sur les poissons fossiles. T. v, pp. 93-97; Atlas, table F, fig. 1; table x, figs 1, 2, and 3.

Aldrovandi, Ulyssis. 1613. De Piscibus libri V et de cetis liber unus. Pages 100–103, figure.

Aristotle. Historia Animalium. Book ix, chapter 3; 610*b*, 5 (D'Arcy W. Thompson trans., vol. iv of complete works, 1910).

Brenchley, Julius L. 1873. Jottings during the cruise of H. M. S. *Curaçoa* among the South Sea islands in 1865, p. 261 and frontispiece. London.

Browne, Patrick. 1756. The civil and natural history of Jamaica, p. 443.

Büttikofer, J. 1890. Reisebilder aus Liberia, bd. ii, p. 45, Leiden.

Bullen, Frank, T. 1904. Denizens of the deep, pp. 310–328.

Catesby, Mark. 1754. The natural history of Carolina, Florida, and the Bahama Islands. Vol. ii, p. i, and table i.

Chisholm, C. 1808. On the poison of fish. Edinburgh Med. and Surg. Jour., vol. iv, p. 394.

Cuvier and Valenciennes. 1829. Histoire Naturelle des Poissons. T. iii, pp. 240–262, pls. 66–67.

Dampier, William. 1729. Two voyages to Campeachy. Vol. ii; part ii of Dampier's Voyages, 6th ed. (Masefield's edition, 1906), p. 172.

Day, Francis, 1865. The fishes of Malabar, p. 64. London.

Duméril, A. H. A. 1866. Des poissons vénéneux. Ann. Soc. Linn. Départ. Maine-et-Loire, vol. viii, pp. 1–17.

———. 1867. On venomous fishes. Annals and Mag. Nat. Hist., 3 ser., vol. xx, p. 153–167. [Translation of above.]

Du Tertre, R. P. 1667. Histoire Générale des Antilles. T. ii, pp. 204–206.

Evermann, Barton Warren, and Millard Caleb Marsh. 1900. The fishes of Porto Rico. Bull. U. S. Fish Comm. for 1900, vol. xx, pp. 115–116, fig. 26.

Felix, Johannes Paul. 1890. Beiträge zur Kenntniss der Gattung Protosphyræna Leidy. Zeitsch. d. deutsch. geologischen Gesellschaft, bd. xlii, pp. 278–302, tafel xii.

Ferguson, William. 1823. On the poisonous fishes of the Carribbee Islands. Trans. Roy. Soc. Edinburgh, vol. ix, p. 72.

Fermin, Philippe. 1769. Description generale, historique, geographique et physique de la colonie de Surinam, pp. 254–255. Amsterdam.

Fischel, Friedrich, and Carl Enoch. 1892. Ein Beitrag zu der Lehre von den Fischgiften. Fortschritte der Medicin, Bd. x, pp. 277–290.

Fowler, Henry W. 1903. New and little-known Mugilidæ and Sphyrænidæ. Proc. Acad. Nat. Sci. Philadelphia, vol. lv, p. 75.

Gesner, Conrad. 1558. Historiae Animalium. Liber iiii, De Piscium & Aquatilium Animantium Natura, pp. 1059–1062, figure.

Gill, Theodore. 1862. Remarks on the relations of the genera and other groups of Cuban fishes. Proc. Acad. Nat. Sci. Philadelphia, vol. xiv, pp. 239–240.

Gosse, P. H. 1851. Natural History. Fishes, p. 72.

Gudger, E. W. 1912. Natural history notes on some Beaufort, N. C., fishes, 1910–11. No. I, Elasmobranchii—with special reference to utero-gestation. Proc. Biol. Soc. Washington, vol. xxv, p. 142.

Günther, Albert C. L. 1877. Andrew Garrett's Fische der Südsee. Heft vi, [in] Jour. des. Mus. de Godeffroy, Heft xiii, p. 211.

Guichenot, Alphonse. 1853. Poissons (in Sagra, Ramon de la., Histoire Physique, Politique et Naturelle de l'Ile de Cuba, tome ii, pp. 37–42. Paris).

Haweis, Stephen. 1917. The book about the sea-gardens of Nassau, Bahamas, p. 78. New York.

Henderson, John B. 1916. The cruise of the *Tomas Barrera*, p. 51.

Holder, Charles Frederick. 1903. The big game fishes of the United States, pp. 88–98.

———. 1908. Big game at sea, pp. 282–286.

———. 1910. The Channel islands of California, p. 125.

Jordan, David Starr, and Barton Warren Evermann. 1896. The fishes of North and Middle America. Vol. i, p. 823; vol. iv, 1900, pl. cxxviii, fig. 349.

———, and Joseph C. Thompson. 1905. The fish fauna of the Tortugas Archipelago. Bull. Bureau of Fisheries for 1904, vol. xxiv, p. 236.

Labat, R. P. 1742. Nouveau Voyage aux Isles de l'Amérique. Vol. ii, pp. 30–36.

Leidy, Joseph. 1860. Remarks on *Saurocephalus* and its allies. Trans. Amer. Philos Soc., vol. xi, p. 91.

Linton, Edwin. 1908. Helminth fauna of the Dry Tortugas. Carnegie Inst. Wash. Pub. No. 102, pp. 160, 162, 164.

———. 1910. Helminth fauna of the Dry Tortugas; ii Trematodes. Carnegie Inst. Wash. Pub. No. 133, pp. 17, 19, 81.

Linstow, O. von. 1906. Neue und bekannte Helminthen. Zoologischer Jahrbücher (Syst. Abth.), 1906–1907, vol. xxiv, pp. 1–2.

L[ocke], J. 1675. Extract from a letter written to the publisher about poisonous fish in the Bahama Islands. Philos. Trans. Roy. Soc., vol. x, p. 312.

Lütken, Chr. 1878. Ichthyografiske Bidrag. VII. Tillægsbemærkninger om sugefiske og sværdfiske, Vid. Meddel. Naturhisk. Foren. Kjobenhavn, 1877-1878, ser. 3, vols. ix and x, pp. 242–243.

Macgillivray, John. 1852. Narrative of the voyage of H. M. S. *Rattlesnake*. Vol. i, p. 69.

Moreau de Jonnes, Alexandre. 1819. Recherches sur les poissons toxicofères des Indes occidentales. Bull. Soc. Philomath. Paris, 1819, p. 136.

———. 1821. Recherches sur les poissons toxicophores, pp. 1–34. Paris.

Morton, Charles. 1868. [Diarrhoea as a result of fish poisoning at Nassau, Bahamas.] Statistical Report Health of Navy for year 1865, pp. 107–108. London.

Mowbray, L. L. 1916. Fish poisoning (Ichthyotoxismus). Bull. N. Y. Zool. Soc., vol. xix, pp. 1422–1423.

Parra, Antonio. 1787. Descripcion de differentes piesas de historia natural las mas del ramo maritimo [Cuba], pp. 90–92.

Pellegrin, Jacques. 1900. Les Poissons Vénéneux. 121 pp., 16 figs. [Reviewed in Revue Scientifique, ser. iv, vol. 14, pp. 178–180.]

Pike, Nicholas. 1873. Sub-tropical rambles in the land of the Aphanapteryx [Island Mauritius], pp. 286, 294.

Plinius Secundus, C. Naturalis Historium; Book xxxii, chapter 11, paragraph 54. [D. Detlefson ed. and trans. 1873.]

Poey, Felipe. 1856–1858. Memorias sobre la historia natural de la Isla de Cuba. Tomo ii, pp. 164, 165, 265.

Reeve, Henry F. 1912. The Gambia, its history, etc. A description of the birds, beasts, and fishes found therein, p. 273. London.

Rochefort, Charles de. 1665. Histoire naturelle et morale des Iles Antilles de l'Amérique, page 197, figure.

Rondelet, Guillaume. 1558. La première (et seconde) partie de l'histoire entière des poissons, pp. 184–185, figures.

Salviani, Hippolyte. 1554. Aquatilium Animalium Historia, Liber primus, cum eorundum formis, aere excusis. Romæ, pp. 70–71, plate viiii.

Saville-Kent, W. 1893. The Great Barrier Reef of Australia, p. 293.

Sloane, Hans. 1707. A voyage to . . . Jamaica with the natural history of [that island]. Vol. i, pp. 23–24, 28–29; vol. ii, pp. 285–393.

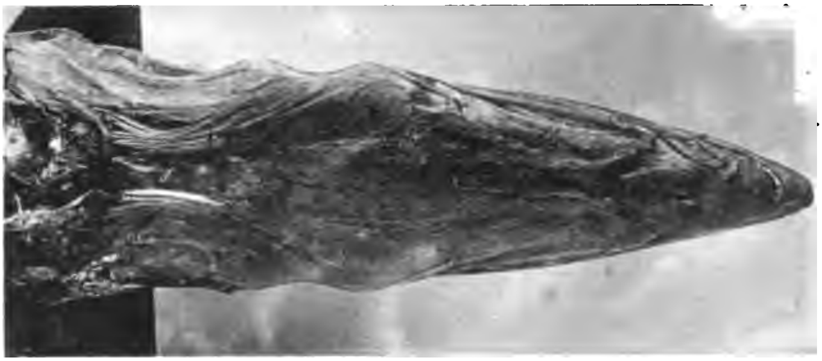
Temminck, E. J., and H. Schlegel. 1850. Poissons (in Siebold, Ph. Fr., Fauna Japonica, pp. 33–34). Lugduni.

Vincent, Harry. 1910. The sea fish of Trinidad, pp. 27–28, 70.

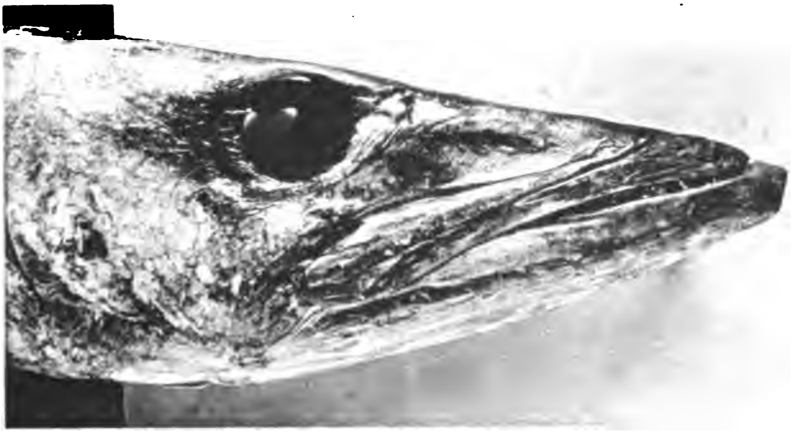
Ward Thomas. [Paul Fountain, editor.] 1907. Rambles of an Australian naturalist, pp. 125, 313.

Weber, Max. 1913. Die Fische der Siboga-Expedition, p. 150.

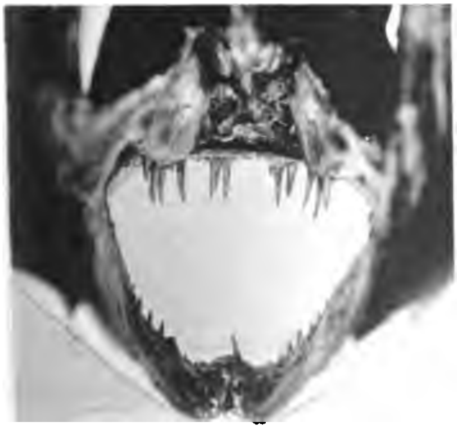
Wood-Jones, F. 1902. Corals and atolls, p. 322, figure.

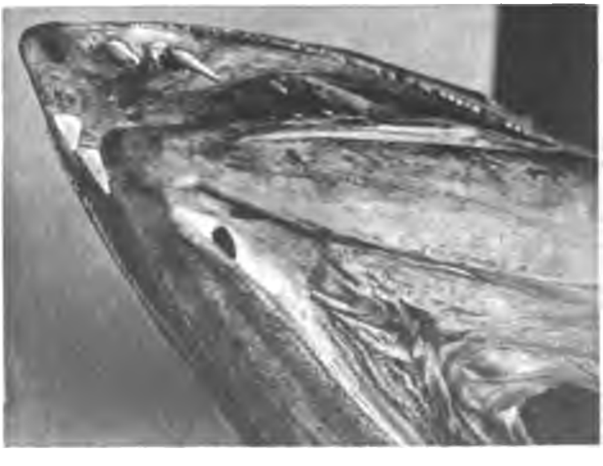


3



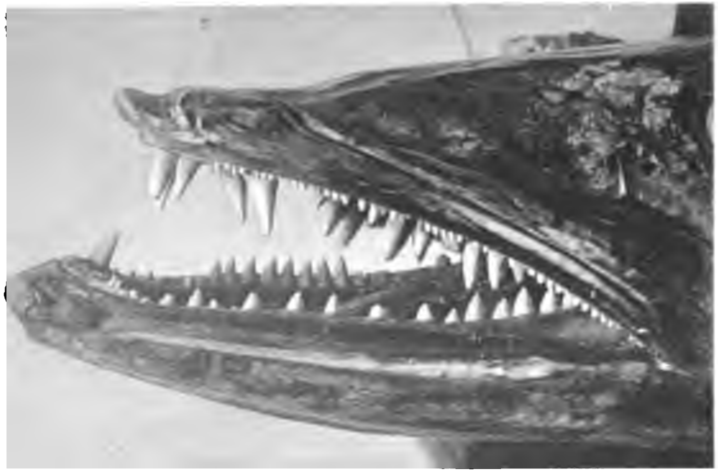
4



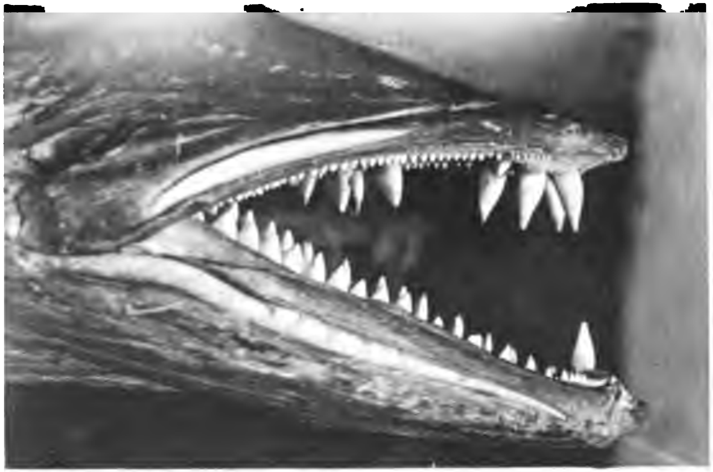


6

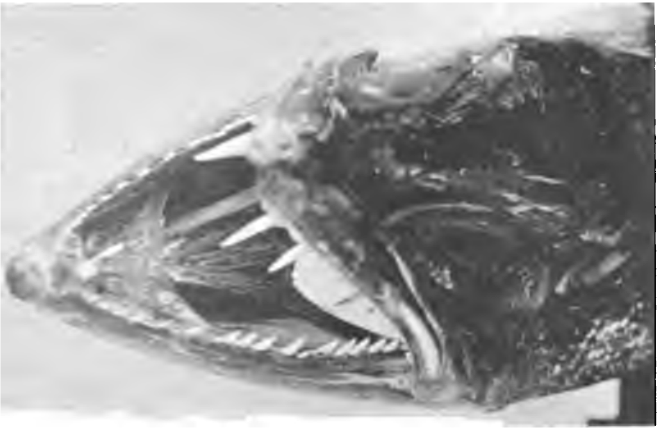
3. Head of *S. barracuda* seen from above, fresh specimen.
4. Lateral view of same head.
5. Looking into mouth of barracuda from behind, Miami specimen.
6. Under side of dried head showing apertures behind point of lower jaw, and also socket in apex of upper jaw for reception of solitary mandibular fang.



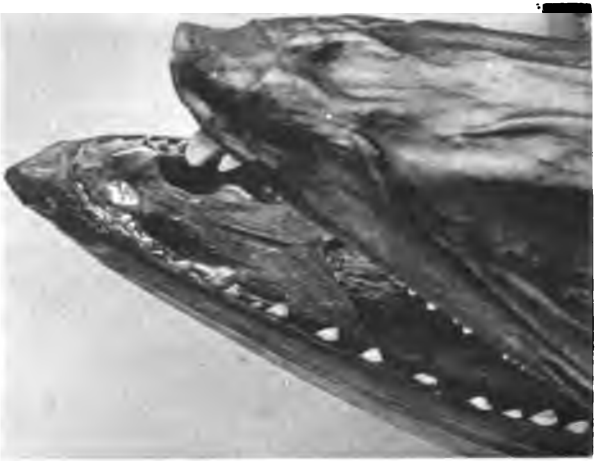
7



8

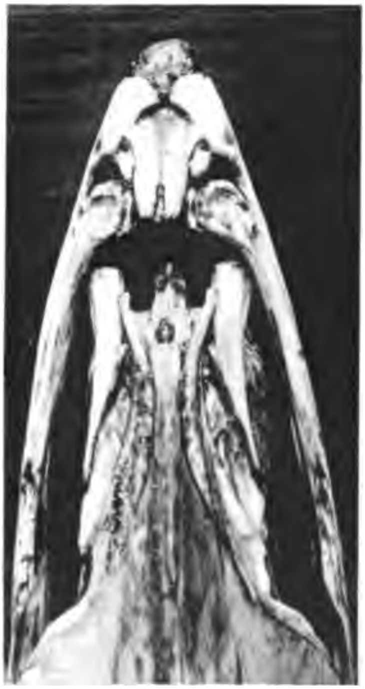


9

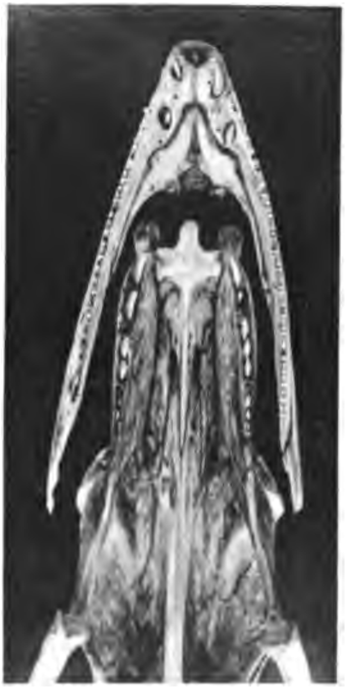


10

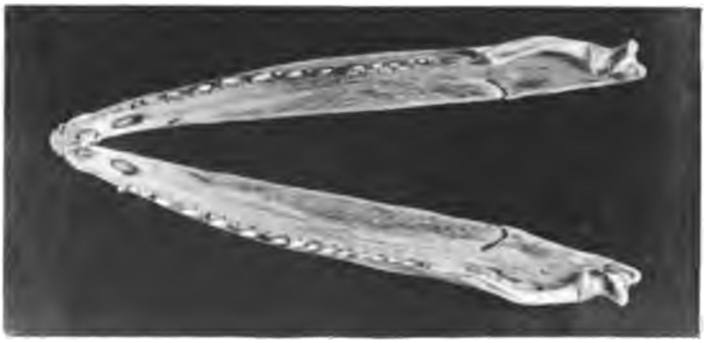
7. Mouth and jaws of 55-inch barracuda, dried head.
8. Mouth and jaws of fish figured in Plate I, dried head.
9. Mouth of *S. barracuda* showing teeth, tongue, mandibular breathing valve and spaces to receive palatine teeth — dried head of fish figured in Plate I.
10. Mouth showing teeth and mandibular breathing valve with depressions to receive great fangs of upper jaw. From same head shown in figure 7, this plate.



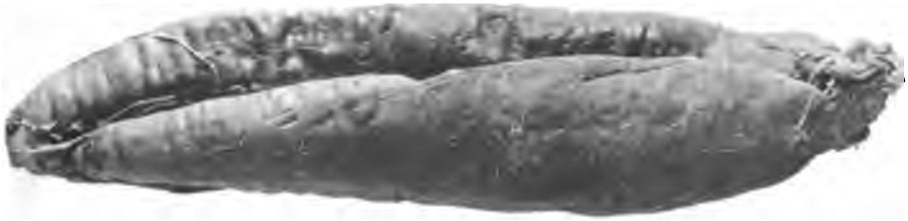
11



12

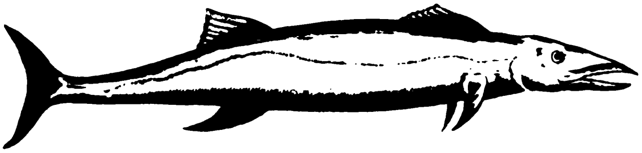


13

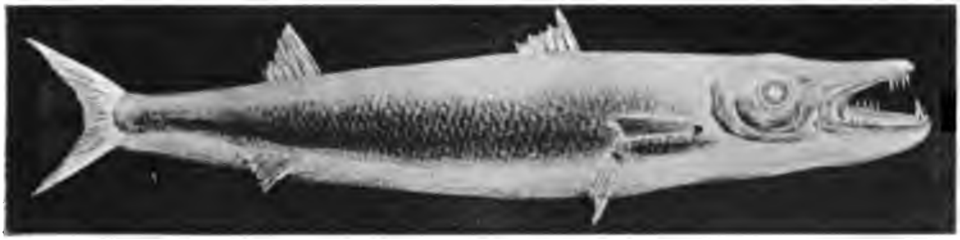


14

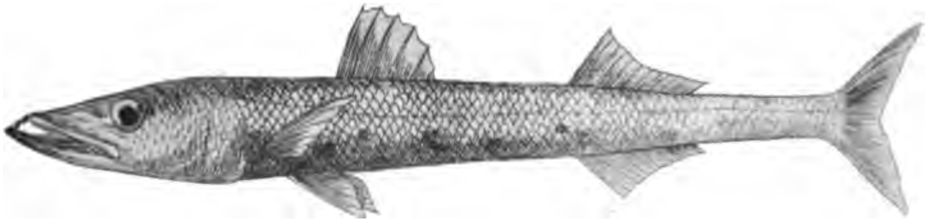
11. Dorsal view of skull with upper jaw displaced forward showing joint between the two.
12: Ventral view of the same.
13. Lower jaw showing teeth and also sockets in mandibular bones for reception of premaxillary fangs.
14. Ovary of 55-inch barracuda.



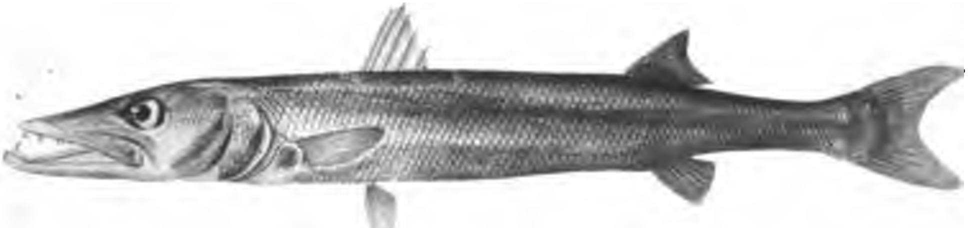
15



16

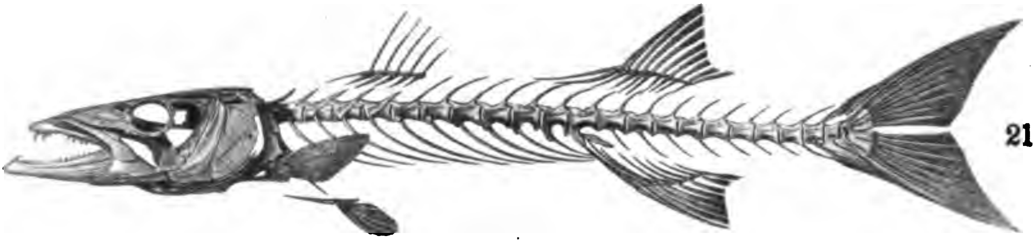


17

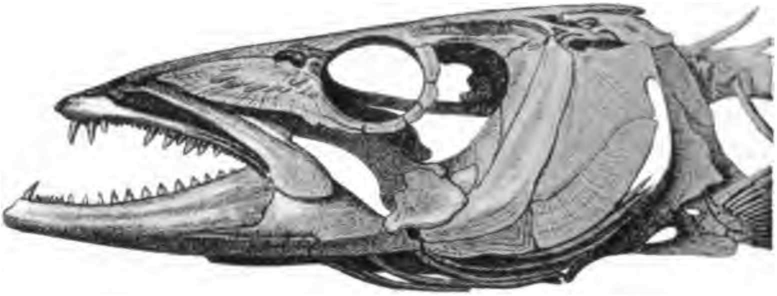


18

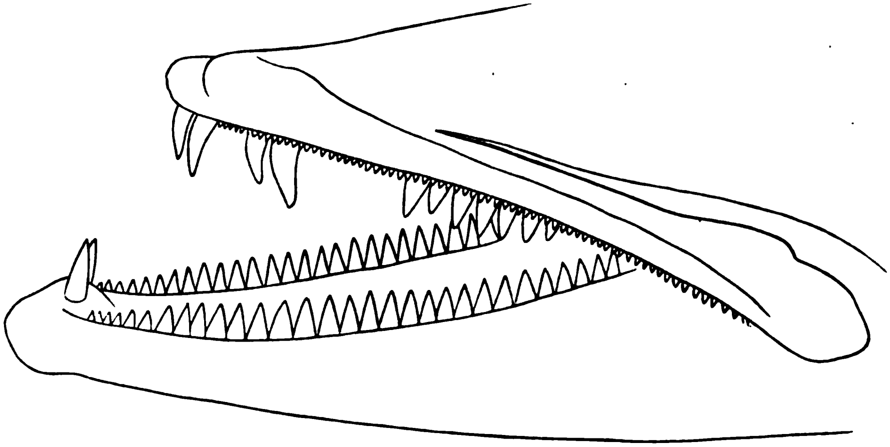
15. Becune, after Rochefort, 1665.
16. Barracuda, after Catesby, 1754.
17. Picuda, after Parra, 1787.
18. Sphyrène, after Cuvier and Valenciennes, 1829.



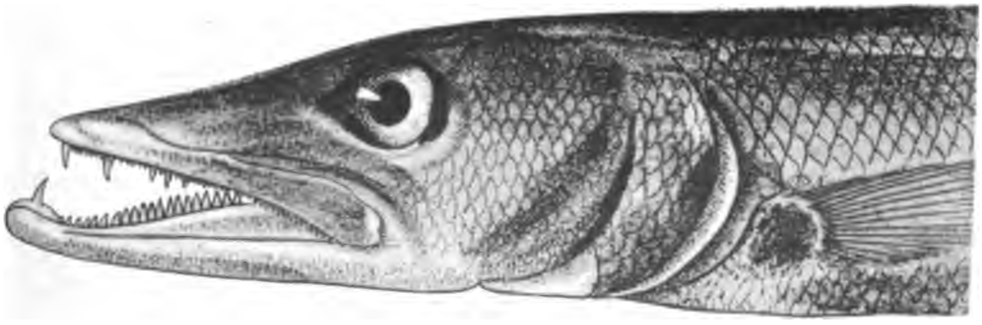
21



22

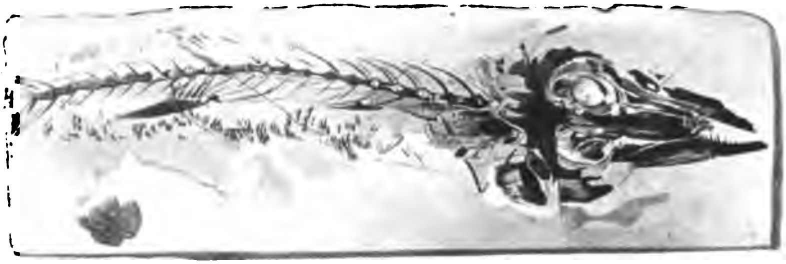


19

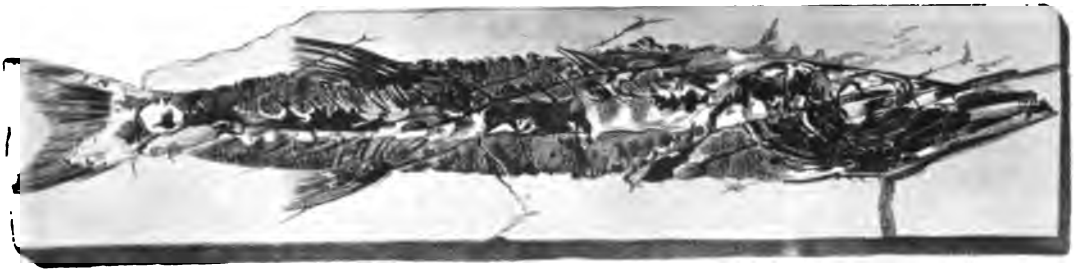


20

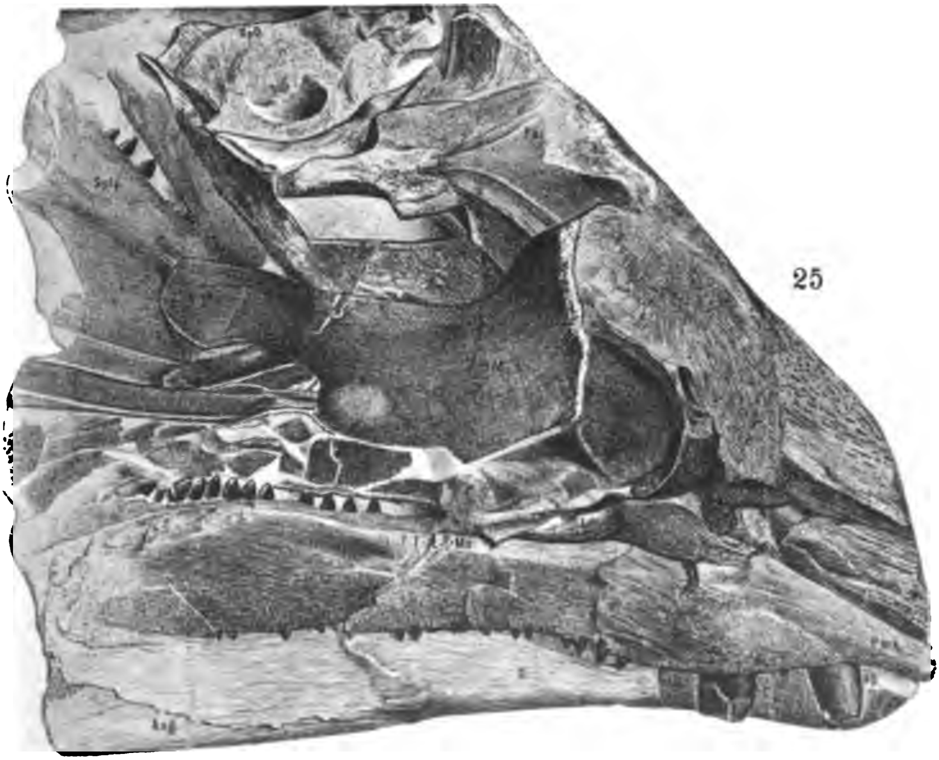
19. Much enlarged line drawing of head from Cuvier and Valenciennes.
20. Enlarged head from Cuvier and Valenciennes, colored figure, plate V, figure 18.
21. Skeleton of *S. barracuda* after Agassiz, 1843.
22. Head of the same much enlarged.



23



24



25

23. *Sphyræna gracilis*, after Agassiz.
24. *Sphyræna bolcensis*, after Agassiz.
25. *Protosphyræna nitida*, after Felix.

V.

BOTANICAL ECOLOGY OF THE DRY TORTUGAS.

BY H. H. M. BOWMAN
Of the University of Pennsylvania

Six plates and seven text-figures.

BOTANICAL ECOLOGY OF THE DRY TORTUGAS.

By H. H. M. Bowman.

As the name of these islands indicates, their vegetation is characteristically xerophytic, although the rainfall is sufficient to assure the plants the necessary amount of water. Living in such a difficult environment, as may be supposed, the plants are very interesting when a close study is made of their individual characteristics. The opportunity for such study was given the writer during the summers of 1915 and 1916, while pursuing another line of botanical research under the auspices of the Carnegie Institution of Washington, which partially controls one of them (Loggerhead Key), where a marine laboratory is maintained.

The Dry Tortugas are situated in the Gulf of Mexico between 24° 34′ and 24° 42′ north latitude and between 82° 48′ and 82° 58′ west longitude. The relative distances in the cardinal directions of points on land are as follows: approximately 920 miles east of Tampico, Mexico; 625 miles southeast of New Orleans; 470 miles south by east of Pensacola; 70 miles west of Key West, and 90 miles north by west of Havana. The Tortugas are really the westernmost of all the Florida Keys, but are more detached from them and have different geological and botanical aspects.

GEOLOGIC FORMATION.

The geological formation and the history of south Florida and the adjacent regions have been investigated thoroughly by T. Wayland Vaughan, of the United States Geological Survey, and in one paper he discusses particularly the Tortugas Atoll.[1] From this paper the writer quotes largely as to the geology of these islands. The Dry Tortugas consist of eight small islands: Loggerhead Key, Bird Key, Garden Key, Long Key, Bush Key, Sand Key, Middle Key, and East Key; these, together with large submerged banks and several shoals which were, until recent hurricanes, charted as islands, form an irregular ellipse with its longer axis directed from northeast to southwest. The lagoon inside of this atoll has a depth of 5 to 7 fathoms.

As stated in Vaughan's interesting account, two lines of investigation were taken up in determining the geology and origin of the Tortugas atoll: first, whether submarine solubility phenomena played an important part in their history; second, whether the wind and current action were responsible. To determine the first, a chemical research was conducted into the dissolving power of sea-water due to the CO_2 it contained, and the material from the bottom of the lagoon was examined. This latter examination showed the bottom to be fine calcareous mud, precipitated by denitrifying bacteria, as discovered by Drew.[1] The former chemical research was made by Dole[2] on daily observations of the amount of CO_2 in the sea-water flowing into the lagoon and the amount of carbonates in the water flowing out. The result of this work showed that the dissolving action of CO_2 in the water was negligible and from the bottom samples it was ascertained that deposition was going on at a rate far exceeding any solubility.

---

[1] Vaughan, T. Wayland, The Building of the Marquesas and Tortugas Atolls and a Sketch of the Geologic History of the Florida Reef Tract. Carnegie Inst. Wash. Pub. No. 182, pp. 55–67.

The above study eliminated the first theory of action by solubility, but the theory of formation by waves and currents remained. As observed by various writers, sand dunes and water-carried detritus are alike molded into two shapes by certain wind and water currents. The latter are formed by an obstacle or a counter-current shearing a constant current, which drifts material to both sides and a crescent is formed with the bow of the arc facing the current. Vaughan further says that there are three kinds of currents working in this region: first, wind-formed currents, accompanied by waves; second, the Florida counter-current; third, tidal currents. The prevailing wind in the Tortugas is from northeast to southwest, and the islands also lie in the sweep of the Florida counter-current, which moves west, so that these two sorts of currents cooperate; on the other hand, the general tidal current is from north to south—*i. e.*, flowing transversely to the two before mentioned; thus the arc of the Tortugas Atoll is bowed toward the east, against the prevailing wind and the counter-current. The southwest part of the perimeter trails along the direction of these currents. The southwest and southeast passages in the atoll are attributed to the influence of the counter tidal current. White Shoal and Brilliant Shoal indicate the direction of this current, while Loggerhead, the largest of the group, shows the elongated "linear-ridge" shape due to the deposition of material by currents trailing along from the tails of the crescent, and the indurated beach rock on this island shows it to be wave-built, by its seaward slope.

On Garden Key, as seen by borings made by Vaughan, the larger rock-masses were found to be massive dead coral-heads. All of the Tortugas Keys are composed of calcareous detritus, the remains of various $CaCO_3$-secreting organisms, mollusks, corals, nullipores, echinoderms, and the calcareous algæ. The coral fauna of the Tortugas, which has also been extensively studied by Vaughan, is quite rich and has contributed a great deal to this detritus. The atoll is therefore formed by certain currents arranging this loose calcareous detritus. This material above sea-level is unconsolidated, but below 8 feet indurated beach-rock is found. This constituted an older formation for the growth of reef corals. "It is probable," Vaughan says, "that this rock was sub-aerially indurated and then depressed—*i. e.*, the atoll was outlined previous to coral growth in this region." The whole of the south Florida region was deposited in Pliocene times during depression; then in Pleistocene time there was an uplift and again a depression with a succeeding uplift. During the Pleistocene period it is supposed that some of the coral reefs stood as much as 18 feet above sea-level. In recent times there has been a depression, which, however, has left the keys slightly higher than they were before the Pleistocene depression. The evidence of these oscillations is borne out by the knowledge of the growth habit, in relation to sea-level, of the reef-forming corals in the Florida region.

---

[1] Drew, G. H., On Precipitation of $CaCO_3$ in the Sea by Marine Bacteria and an Account of Denitrifying Bacteria in Tropical and Temperate Seas. Carnegie Inst. Wash. Pub. No. 182, pp. 7–45.

[2] Dole, R. B., Some Chemical Characteristics of Sea-Water at Tortugas, Florida. Carnegie Inst. Wash. Pub. No. 182, pp. 69–78.

CLIMATIC CONDITIONS OF THE TORTUGAS.

While no permanent records are available from the Tortugas, the writer assumes that the records from the office of the United States Weather Bureau at Key West, only 68 miles distant, will for this purpose be applicable to the climate of the Tortugas. The records in this office have been kept since 1871 and are very reliable.

For botanical consideration the subject of rainfall is quite important. According to the Key West records the total average rainfall is 38.66 inches. The precipitation is quite varied and depends largely in summer on tropical squalls which suddenly appear on the horizon and sweep over the islands with a fierce rush of wind and driving torrents of rain. These perhaps last only 20 minutes to an hour. The maximum total precipitation for 24 hours, for the year 1914, for instance, was 4.80 inches, which occurred in November of that year. An idea of the variation may be gained by comparing this with the maximum precipitation for 24 hours in January of the same year, which was only 0.31 inch. It may be mentioned in this connection that the greatest precipitation in this region occurs during the months of September, October, and November. As stated above, the average annual rainfall for Key West (and presumably the approximate for Tortugas) is 38.66 inches. By comparing this with the total average precipitation for Miami, situated on the mainland of Florida, 125 miles north of Key West, which is 46.56 inches, it is seen that the average is slightly lower for the southern keys than for the main peninsula.

The average number of clear days for these southern keys is 151. During the summer months of May, June, and July the writer has lived in the Tortugas, when for periods of several weeks there was a continuous succession of clear, brilliantly sunny days with not even an occasional tropical storm or squall. According to the meteorological summary for Miami, the total average of clear days is only 68 and partly cloudy 135. The comparison again, with the record for Key West, with its average of 151 days, taken together with the difference in total precipitation, shows the climate of the southernmost keys to be slightly drier than that of the mainland. This difference is perhaps due largely to the prevailing winds.

The prevailing winds are eastwardly, as reported by the Weather Bureau at Key West, and as also observed at Tortugas by Vaughan, in his paper on the influence of the prevailing wind in atoll formation, and also by the writer in various physiological experiments undertaken at the latter place. The velocity of these winds is very variable. The average velocity is 9.6 miles per hour. But in this region of hurricanes all winds over 75 miles per hour are rated as hurricane winds. These usually occur in the fall during the months in which the heaviest precipitation occurs, viz, September, October, and November, which months are called the "hurricane months." In the Gulf region, as recorded by the Key West office, the following severe hurricanes, of which the directions and velocities are here given, did a great deal of damage: On September 25, 1894, 87 miles per hour from the southwest; October 19, 1896, 88 miles per hour from the southwest; October 11, 1909, 83 miles from the northeast; October 17, 1910, 100 miles per hour from the south. This last hurricane, known as the "great hurricane of 1910," not only did vast damage on land but, as observed by the writer and others by means of a diving helmet and glass-bottomed boats, the coral and sponge fauna and the algal flora of the sea-bottom in the region suffered great changes and in some places on the bottom immense windrows of broken corals may still be seen which were mashed and heaped up by the force of giant wind-driven waves.

The temperature of the Tortugas is fairly constant. The variations in January and February, which are the coolest months, range from 60° F. as a minimum for these months to 75° F. as the maximum; in June, July, and August, which are perhaps the hottest months, the minimum is 77° F. and the maximum 88° F. The Key West records show the average annual temperature to be 76.8° F. and for the entire period over which the records extend—*i. e.*, 1871 to 1913 inclusive—the maximum was 100° F. and the minimum 41° F. For Miami the mean annual maximum temperature is 80° F. and the minimum is 68° F.; that is, the annual mean temperature is slightly lower than that of the Tortugas and the southern keys.

GENERAL SKETCH OF THE VEGETATION OF THE TORTUGAS.

On ecologic and geologic grounds the Florida Keys have been divided into four groups by Small:[1]

(1) The upper sand keys, which are really detached portions of the coastal peninsula and support a sand-dune and hammock flora closely related to the mainland.

(2) The Upper Keys, extending from Soldier Key to Spanish Harbor, and which are composed of a coral rock. These have a vegetation of tropical hardwood trees and shrubs and palms resembling those of the Bahamas which lie slightly to the east of them.

(3) The Lower Keys from No Name Key to Key West. These have a basic formation of Miami limestone. The flora of the group, according to Small, who has done much systematic work in this region, is quite varied, having large areas of pineland and palms as well as extensive hammocks. This flora is more closely related to Cuba, which lies only 90 miles to the south of this group.

(4) The group most directly considered in this paper is called the Lower Sand Keys. They are all the keys lying west of Key West and are composed of sand or, more strictly, according to the above analysis of Vaughan,[2] of coarse calcareous detritus, the remains of various organisms. Of these Lower Sand Keys Small[1] says "they are little more than sand bars and they support, like the ocean side of all the Florida Keys, only or mainly the characteristic strand flora of most of the West Indies."

The origin and relationship of the Tortugas flora may thus easily be traced to the adjacent large islands of the Antilles, Cuba, etc. The majority of the plants in the Tortugas are easily transported by the sea, as indeed are most strand floras.

A typical ecological formation in the Florida Key region is the mangrove association, *Rhizophora mangle*, but this association is entirely lacking in the Tortugas, owing to the physical nature of the islands. In the writer's work with *Rhizophora*, he observed that the floating hypocotyls must have a secure anchorage, either in the deep, soft mud amid the entangled roots of a mangrove swamp, or in the clefts and cracks of a coral rock, a mud-flat, or oolite bottom. The same plummet-like action of a young mangrove hypocotyl in boring a resting-place for itself in soft mud, or in finding a cleft by its twirling action in the water-currents, was observed by Crossland[3] on the Zanzibar reefs. The sand beaches of the Tortugas do not furnish the required conditions for the young viviparous seedling. The lack of sufficient moisture in the coarse, porous sand, together with the dry winds, soon kills the drifted young mangrove. This has been demonstrated many times in the physiological experiments of the writer on *Rhizophora* and explains the absence of the mangrove association in the Tortugas.

---

[1] Small, J. K., Flora of the Florida Keys. 1913, III–IV.

[2] Vaughan, T. Wayland, The Building of the Marquesas and Tortugas Atolls and a Sketch of the Geologic History of the Florida Reef Tract. Carnegie Inst. Wash. Pub. No. 182, pp. 55–67.

[3] Crossland, C., Note on Dispersal of Mangrove Seedlings, Annals of Botany, XVII, p. 267.

The plants of the Tortugas, living as they do in such uniformly xerophytic conditions and on such small areas of land, are naturally confined to a small group called a strand flora, on account of characteristic species which occur on all maritime shores and particularly tropical coasts—such genera as *Scævola*, and even the species *Ipomœa pes-capræ* as found on all tropical beaches, as far as the remote islands of the Pacific, according to Guppy[1] and Schimper;[2] the latter, in his Plant Geography,[3] in his classification of tropical littoral formations calls the open sandy formation a "pes-capræ formation"; Harshberger[4] also includes a great many species found scattered all over the Tortugas in his treatment of sea-strand formations in South Florida, from which, however, the Florida Keys are excluded.

Broadly, it may be said that the entire flora of the Tortugas is a strand flora, but to the close observer these plants are easily seen to fall into certain associations or groups of several species. The reason for this grouping of species in a fairly uniform flora the writer believes to be due to two factors: first, the prevailing winds, which frequently carry dense salt spray or mist inshore, and certain of these strand plants are better able to withstand this drenching with a rather strong solution of sodium chloride and magnesium and calcium carbonates, of which latter salts the sea-water contains, in this region, fairly high percentages;[5] second, the aggressiveness of certain species and the ability to hold a position occupied by them against invading species—for instance, few plants will advantageously invade an area occupied by *Opuntia*, even though the surface is not entirely covered by its sprawling joints.

The writer, then, recognizes four fairly marked associations in the general Tortugas flora of strand plants: (1) the *Uniola* community; (2) the *Suriana* community; (3) the *Opuntia* community; (4) the *Chamæsyce* community.

The first association, the *Uniola* community, is rather heterogeneous and varies slightly on different beaches, depending on the conditions in rough weather and the prevailing winds. The lowest on the beach and close to the high-tide limit is *Cakile lanceolata* (Wildenow) O. E. Schulz. The dried stalks of this may be seen in July, and during August they are uprooted and blow about the beaches as tumble-weeds. Growing with *Cakile* is the sand-spur, *Cenchrus incertus* M. A. Curtis, which forms large flat mats on the beaches, bearing its spikelets in a heavy spiny involucre, making a painful wound if stepped upon. Associated with this in dense masses the sea purslane (*Sesuvium portulacastrum* L.) is often seen; resembling it superficially is *Alternanthera maritima* St. Hildmann. Back from the reach of spray is *Sporobolus virginicus* (L.) Kunth; frequently out in line with it is *Tournefortia gnaphalodes* (Jacquin) R. Brown, forming large characteristic, flat-topped bushes 1 to 1.5 meters tall, clothed with soft, whitish-gray, tomentose leaves. The long, trailing vines of the purple beach morning-glory, *Ipomœa pes-capræ* (L.) Sweet, are found here, and also *Scævola plumieri* Vahl, in clumps with thick, glossy leaves, nauseating black berries, and white blossoms with the peculiar corolla split down one side and the odd ciliated indusium on the stigma. This last is one of the most singular of all the plants of the region. On the higher portions of the beaches and a meter or two back from the water-line, sea oats, *Uniola paniculata* L., will be observed, a grass about 2 meters tall, with a gracefully drooping panicle of heavy spikelets. This grows in rather close formation over large areas, forming thick tufts as it grows from season to season, and the dead culms remain clustered about the living ones.

---

[1] Guppy, H. B., Observations of a Naturalist in the Pacific. 1913.

[2] Schimper, A. F. W., Die Indo-Malayische Strandflora.

[3] Schimper, A. F. W., Plant Geography upon a Physiological Basis. 1903, p. 387.

[4] Harshberger, J. W., The Vegetation of South Florida. Wagner Institute of Science, Philadelphia, 1914, vol. VII, pt. 3.

[5] Mayer, A. G., Annual Report of Director of Department of Marine Biology, Carnegie Inst. Wash., 1910, Year Book No. 9, p. 122, quoting from Report on Analysis of Tortugas Sea-Water, by Division of Physical and Chemical Research, U. S. Geol. Survey.

The most important association in the *Suriana* group covering very large tracts, is formed by the bay cedar, *Suriana maritima* L., a shrub of the Geraniales order, 1 to 2 meters tall. The deep green of this shrub forms a pleasant and lively contrast to the shore plants covered with gray tomentum, as, for instance, *Tournefortia*. With *Suriana*, and often climbing up over it and hiding its small yellow star-shaped blossoms, is the beach bean, *Canavalia lineata* (Thunberg) De Candolle. A common epiphyte on the lower branches of the *Suriana* bushes is the lichen *Usnea barbata* L., with its small gray branches sticking out like a sort of a mossy covering on the twigs.

The third association is the *Chamæsyce* group, which also covers large areas on some of the keys, its members growing in situations not so suitable for the previous group. The chief component of the association is *Chamæsyce buxifolia* (Lamarck) Small. This spurge forms tracts thickly covered with its small gray-green boxwood-like leaves and tough brown stems filled with abundant latex. The rooting system of the plant is remarkable; a small individual, only 3 decimeters tall, may have a root system 9 decimeters in diameter, and were the plants growing in a soil less loose and coarse than the Tortugas sand they could scarcely be pulled up by a strong man. An ally of the *Chamæsyce* is a composite, the marsh elder, *Iva imbricata* Walter, which forms a rather tall bush, about 10 to 15 decimeters, with small, light-green succulent leaves, which have a rather pleasant bitter, acrid odor and heads of yellowish, inconspicuous flowers. A third member of this group is a sedge, *Cyperus brunneus* S. Watson, forming conspicuous tufts of grayish foliage overtopped by the deep-brown inflorescences.

The fourth association is the *Opuntia* community. This is made up mostly of *Opuntia dillenii* (Ker) Haworth and *Paspalum cæspitosum* Flugge, a rather coarse grass about 4 decimeters tall, the branched inflorescence prolific with dark-brown, disk-shaped seeds. Frequently occurring in this association, and also to some extent in the *Chamæsyce* community, are three species common in the Tortugas: (1) the *Ipomœa pes-capræ;* (2) its more beautiful relative, the white moon-flower, *Calonyction tuba* (Schlechtendal) Colla, which makes a most wonderful effect in the brilliant tropical moonlight with its large salver-shaped corollas starring-over the clumps of *Opuntia* or other supporting vegetation, half hidden in its enveloping heart-shaped foliage; (3) *Melanthera brevifolia* O. E. Schultz, a scrubby composite about 6 decimeters tall, bearing inconspicuous whitish blossoms, but during the hot middle portion of the day they are constantly surrounded by hosts of a tiny butterfly, *Thecla,* whose source of food is the nectar of these little flowers. Into the above four groups the predominating species of the Tortugas may be said to fall naturally on account of the influence of various factors in the environment or those inherent in the plants themselves.

DISTRIBUTION OF SPECIES AMONG THE KEYS OF THE GROUP.

Only one other paper previous to the publication of the present one has dealt with the ecology of this region, viz, a series of maps with field-notes collected by E. O. Lansing in the spring of 1904, and published by Millspaugh in 1907.[1] This work, while fairly accurate and detailed, was compiled from notes evidently made in a very rapid survey of the islands, and in the four days (March 19 to 22 inclusive) allotted to the Tortugas group, the collector naturally overlooked many important minor features in the flora. For instance, evidence of haste is seen in overlooking the groups of sisal hemp, *Agave sisalana* (Engelmann) Perrine, among the bay cedars on Loggerhead, very old plants which were there even in the time of the third predecessor of the present light-house keeper, about 25 years ago; also on the same key numerous clumps and patches of *Opuntia dillenii* among the *Suriana* were not observed. Only one station for this plant is given for Loggerhead, viz, on the west coast near the light-house boat-shed, but the writer found it disseminated fairly well over the island in patches of old plants, often a meter high. These patches have certainly been there since the foundation of the Laboratory of the Carnegie Institution of Washington on the key in 1904, according to statements of Dr. A. G. Mayer, the Director of the Laboratory, and the aged appearance of the plants supplements his statements. But in spite of these discrepancies, the paper has been of great interest to the writer, and, as Millspaugh says in a short introduction,[1] p. 191:

---

[1] Millspaugh, C. F., Flora of the Sand Keys of Florida. Field Columbian Museum Publication 118, Bot. Series II, No. 5.

> "The principal value of the survey lies, therefore, first, in the historical record of the present flora, which should enable future students to determine which species have come to the different islands since 1904 and which have been unable to survive; second, in the knowledge of what species come first to such microcosms, thus forming a basis upon which to judge of the ease or difficulty of dispersion exhibited by certain species, and third, how and in what conformation species spread when brought into an untainted environment."

The paper has thus been of great value, to show what changes have taken place in the flora of the Tortugas in the twelve years which have elapsed since Mr. Lansing's survey—*i. e.*, from 1904 to 1915.

The writer spent the two summers of 1915 and 1916 at the Laboratory in the Tortugas, taking field-notes, making some physiological experiments on the plants, and working on the maps and collections of herbarium material. The herbarium specimens have all been deposited in the Herbarium of the University of Pennsylvania.

The scope of this paper has been confined to the Tortugas group because they are the most isolated of the Florida Keys and their flora is strikingly different from that of the Marquesas, 25 miles to the east, and also from that of the keys to the east of this latter atoll. The most conspicuous feature of this difference is the absence of the mangrove association to any considerable extent in the Tortugas, although Garden Key has a few well-grown young trees which in the summer of the writer's residence in the islands produced flowers and fruits. Another reason was that while the writer had the opportunity and did visit and take notes on the keys above, as well as west of Key West, the longer periods spent in the Tortugas afforded much closer observation and facilitated the securing of experimental data. His work on the other keys was largely or entirely concerned with mangroves, some physiological aspects of which have engaged his attention for several years.

The distribution of species on the various keys of the group is illustrated by maps made in the field by the author with the aid of a plane-table and sighting-rule. The outline map was made in the survey of the group in 1914 and 1915 by Vaughan and Shaw.[2] The various keys will now be taken up separately and the species illustrated by symbols on the distribution maps. A comparison is made in each case with Lansing's survey.

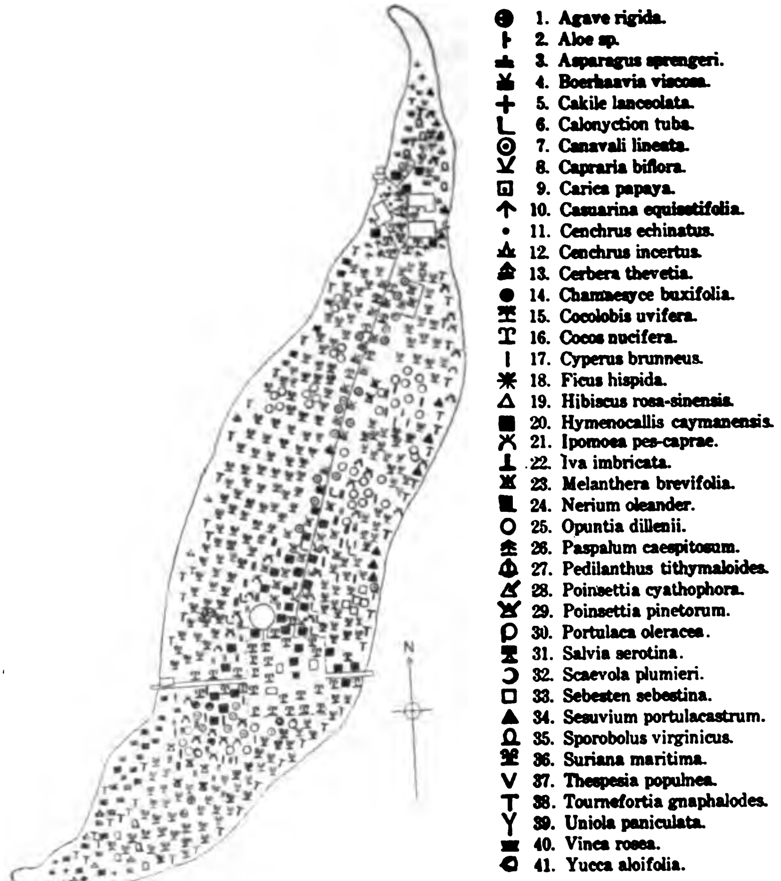
---

[1] Millspaugh, C. F., Flora of the Sand Keys of Florida. Field Columbian Museum, Publication 118, Bot. Series II, No. 5.

[2] Vaughan, T. W., and E. W. Shaw, Geologic Investigations of the Florida Reef Tract. Carnegie Inst. Wash., 1915, Year Book No. 14, p. 232.

LOGGERHEAD KEY.

This is the largest of the Tortugas group and also the highest above sea-level and according to tradition it had in earlier times the largest stand of trees. Old fishermen (and the report was current in the times of the older light-house keepers) state that 75 or 80 years ago this island, as well as Garden Key and others of the group, supported a large stand of old white buttonwood trees, *Conocarpus erectus* L. These were largely cut down by fishermen, who occasionally camped for perhaps several weeks or months in the Tortugas; fires also are said to have had their share in devastating this old silvan flora. The central group of very old and large buttonwood trees in the parade-ground of Fort Jefferson on Garden Key is the only remnant of this supposed aboriginal silvan flora.



Loggerhead Key, showing buildings of the laboratory on the north end and of the light-house on the south. Walks and paths are indicated by lines. One inch = one-fifth statute mile.

The booby, a kind of gannet, is said to have inhabited these islands, but has disappeared with the passing of the trees. Old fishermen relate that men, armed with clubs, would stealthily creep into the groves of buttonwood at night and kill perhaps hundreds of these birds out of pure wantonness, as they had little or no economic value. The same wanton spirit which eradicated the *Conocarpus* groves and the "boobies" in these islands has been shown by the people of the region in regard to the manatee, which is now nearly extinct in these waters; the large green and loggerhead tortoises are also fast disappearing, due to a custom of eating the eggs and killing the females. During the breeding-season of 1916 not over 6 females were reported in these islands as coming in to lay, and two of these were killed after laying and the entire settings of eggs taken, whereas even ten years ago it is said that as many as a dozen females came up on the beach in one night to lay on Loggerhead Key.

The vegetation of Loggerhead is remarkably free from the common tropical weeds when compared, for example, with Garden Key. This is probably because this island has never, since its first permanent occupation 75 years ago, been brought into frequent contact with the mainland by ships, men, or animals, except for a few months shortly after the Civil War, when a quarantine camp was established for the marines brought over from Fort Jefferson during an epidemic of yellow fever. The only permanent residents now are the keepers of the light-house; while the monthly call from Key West of the cutter of the Light-House Establishment, which rarely lasts over 2 hours at Loggerhead Key, does not seem to have served for the introduction of homovectant plants.

A glance at the distributional map of Loggerhead will show that two of the four associations mentioned above are represented in the island, the *Suriana* community predominating and the *Opuntia* association supplementing it. A photograph taken from the top of the light-house shows the vegetation to be distributed in sharply defined areas following irregular outlines. It is supposed that some of the central areas now occupied by the *Opuntia* formation (see plate 3) was cleared of the dense growth of *Suriana* by the marines and soldiers who camped there during the epidemic of yellow fever mentioned above, but the irregular outline and the isolated patches of *Opuntia* among the *Suriana* show that other agencies than man have helped to clear portions of the island.

As remarked before, the *Suriana* does not withstand a drenching of sea-water very successfully, the tomentum of the leaves holding the water until its toxic effect is produced on the leaf-tissue. The great hurricanes of the past must undoubtedly have had their share in cutting out some of the swathes in the *Suriana* community. In the list of species and on the map also some large introduced plants appear, which add conspicuous features to the landscape and perhaps have an influence on the native flora by producing shade and conserving water, etc. Examples of these are the coconut palms, the *Casuarina* trees, the papaws, and the figs planted about the building and gardens of the light-house and laboratory; others are the *Hymenocallis*, the oleanders and *Hibiscus*, *Thespesia*, aloes, yuccas, *Pedilanthus*, and *Asparagus*. All of these, but more particularly the coconuts, papaws, casuarinas, and *Hymenocallis*, have been liberally planted about the laboratory grounds. A noticeable effect of these introduced plants was the increase in the number of plants and the size and vigor of the foliage in *Boerhaavia viscosa* when it grew under these trees or tangled among the *Hymenocallis* plants along a concrete walk leading to the windpump shed at the laboratory. Another example was seen in the greater luxuriance of *Chamæsyce* and *Ipomœa*, when growing near the slight shade thrown by the coconuts, the newer shoots of *Ipomœa* being more slender and tender than when growing in their accustomed habitat on the beach in fierce sunlight. These introduced plants, with the exception of the *Cocos* and *Hymenocallis*, have been brought in and planted by the Director of the Laboratory and have (plate 4) maintained themselves fairly well in such a difficult environment, although only *Casuarina* has reproduced and seeded in. This Australian plant seems to thrive remarkably in these islands. The *Hibiscus* about holds its footing when planted, but *Thespesia* and *Carica* seem to decline if not watered artificially.

An interesting point in a study of the Loggerhead flora is the comparison of Lansing's maps with the present ones. According to Lansing, the island was mainly occupied by bay cedar. Millspaugh also mentions this fact,[1] and states:

> "Its central plateau, like that of Bird Key, is entirely implanted with a pure *Suriana* group, the individuals of which are about 6 feet high. The vegetation of the islet presents no association whatever, all its species being elementally scattered subtropic, maritime 'weeds.' Even the usually associated *Uniola* and *Euphorbia* [*Chamæsyce*] appear as far separated as the limits of the surface will allow."

The author is convinced that Lansing overlooked the old patches of *Opuntia* hidden in the dense growth of *Suriana*, as well as the very old groups of sisal, but aside from this the appearance of the island is much changed in the past 12 years. The spurge, *Chamæsyce buxifolia*, has not changed its position or quantity, but the *Uniola* is now found with it in its station on the northwest corner of the island as well as along the east shore, and very plentifully on the southwest shore. *Iva imbricata* seems now to have disappeared from Loggerhead. *Portulaca*, now as then, is found only around buildings both at the light-house and also at the laboratory. *Melanthera brevifolia* has migrated all along the path leading to the laboratory end of the key and has also spread into the association of *Opuntia,* while *Cyperus* and *Calonyction* occur over the central portion cleared of *Suriana.* The old grove of *Sebesten* (*Cordia*) has not changed in area or spread. *Hymenocallis* is now disseminated fairly well over the island, having been planted largely along all the walks and paths, as well as the coconuts and *Casuarina. Salvia serotina* is found only about the light-house, and *Capraria biflora* is not widely scattered. The shrub *Cerbera thevetia* (which has been planted for the decorative effect of its beautiful yellow blossoms) and the weeds *Poinsettia pinetorum* and *P. cyathophora* were probably introduced with soil brought from the mainland of Florida.

---

[1] Millspaugh, C. F., Flora of the Sand Keys of Florida. Field Columbian Museum Publication 118, Bot. Series II, No. 5.

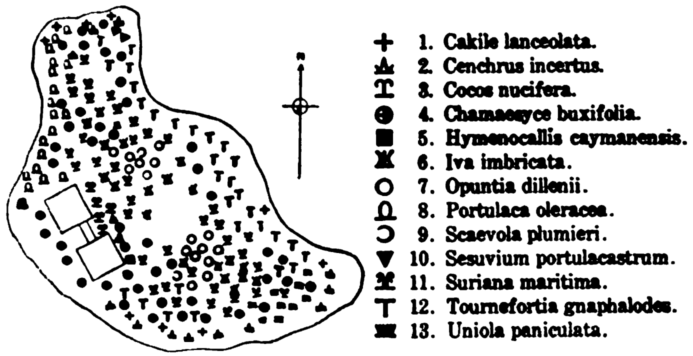
In summarizing the distribution on Loggerhead and comparing it with Lansing's account, the most conspicuous facts are the disappearance of a large amount of *Suriana* from the central portion and the spread into this area of the *Opuntia* group, the increase of *Uniola,* and the disappearance of *Guilandina* (*Cæsalpinia*) *crista, Iva imbricata,* and *Tribulus cistoides.* The appearance of the island is changed on account of the large number of trees and shrubs and other plants introduced for decorative and utilitarian purposes. A comparison of the lists also shows various differences in nomenclature due to synonymy, together with one systematic discrepancy, viz, *Cakile fusiformis,* as listed by Lansing and Millspaugh, is evidently *C. lanceolata* (Wild.) O. E. Schulz. *C. fusiformis,* while indigenous to the keys, has not been noted in the Tortugas. The number of seeds in the pods of the Tortugas species place it as *C. lanceolata.* The total number of species on the island at present is 41, as compared with Lansing's list of 24.

BIRD KEY.

Bird Key, which is considerably smaller than Loggerhead, is only about 500 feet long by 300 feet broad and is now a government bird reservation for the sooty and noddy terns. These birds nest on this small island during the summer months. During the breeding-season for many years this key has been almost covered by these terns, which are now protected by the United States Government and the Audubon Society, and for this purpose a warden is stationed on the island from April to the end of August.

The writer can scarcely estimate the effect produced upon the vegetation of the island by the presence of the birds in such large numbers during the summer and in recent years of the warden, but as the birds live entirely on fish and the warden does nothing to change the physical character of the key these influences do not seem to be of great importance; but since Lansing's survey, in March 1904, a considerable change must have taken place.

Shortly after the Civil War, during the outbreak of yellow fever in Fort Jefferson on the adjacent Garden Key, Bird Key was used as a hospital site and several buildings were erected for the purpose, but we have no means of knowing what plants the key supported in that time, but it was probably during that occupation that *Portulaca oleracea* was introduced, now so prolific on the island. According to Lansing, the island was largely covered with *Suriana*, but if any plant predominates now the author would say it is *Chamæsyce buxifolia*. The *Suriana* is now confined to scattered groups in the northern and southern portions of the island. The center is occupied by masses of *Opuntia* and large quantities of *Chamæsyce* and *Iva imbricata; Cakile* and *Cenchrus incertus* are plentiful along the beaches, particularly of the eastern side. *Tournefortia* is abundant all about the edges of the inner vegetation. It seems to follow the contour of the shore, but keeps back from spray and salt mist. The eastern beach is largely crowded with the gray bushes of *Tournefortia*. *Cyperus* and *Uniola* are found most plentifully on the southeastern tip of the key, but only in tufts and small areas. Around the warden's cottage and the old hospital building are several well-grown coconut palms, which have been planted since Lansing's survey. Near the buildings also are several large clumps of *Sesuvium portulacastrum*, while *Portulaca oleracea* is particularly well scattered on the northwestern shore.



Bird Key, with outlines of old hospital and Bird Warden's cottage.
Two inches = one-fifth statute mile.

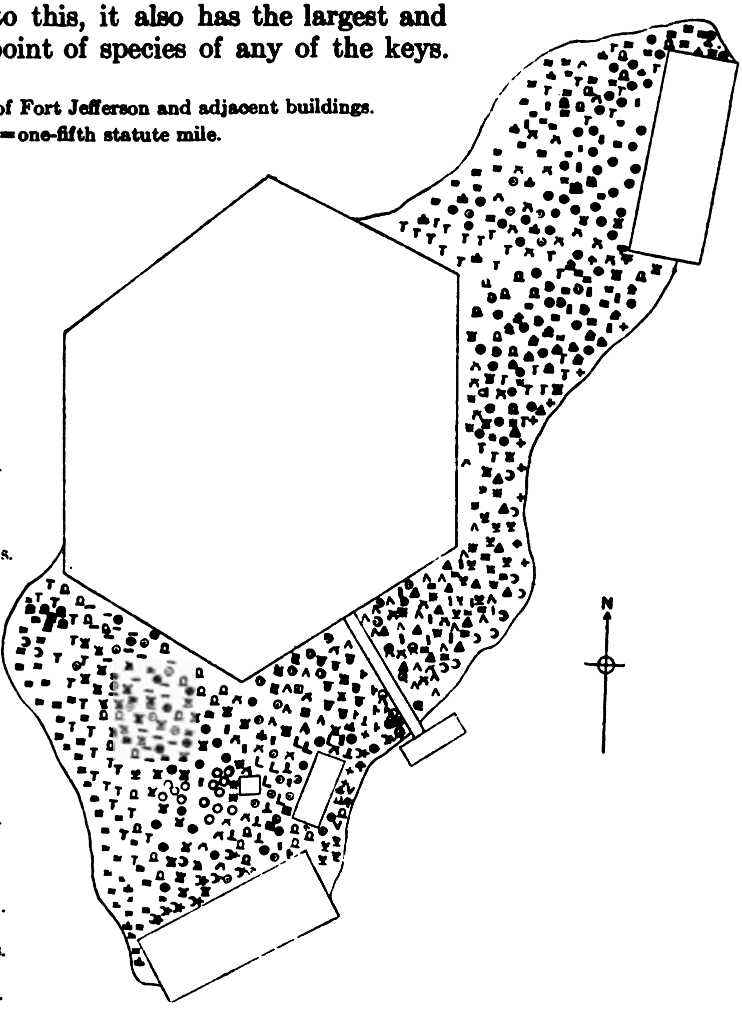
The dominant community on the key is the *Chamæsyce-Iva* association and the noteworthy changes on the island are the ascendency of this group, the decline of the *Suriana* (partly due to storms), the disappearance of *Paspalum*, and the introduction of *Iva*, *Cocos nucifera*, and *Hymenocallis caymanensis*, the latter two plants having been planted by man.

The present number of species for Bird Key is 14, as against Lansing's list of 12.

GARDEN KEY.

This island is the second largest in the group and to a considerable extent is occupied by that antiquated, massive old brick structure, Fort Jefferson (plate 4). Almost all the history and tradition of this remote group of islands centers about Garden Key and the fort. As a fitting corollary to this, it also has the largest and richest flora in point of species of any of the keys.

Garden Key, with outlines of Fort Jefferson and adjacent buildings.
Two inches = one-fifth statute mile.



✱ 1. Alternanthera maritima.
⚑ 2. Argemone leiocarpa.
↑ 3. Atriplex cristata.
丰 4. Avicennia nitida.
+ 5. Cakile lanceolata.
L 6. Calonyction tuba.
⊙ 7. Canavali lineata.
Ͽ 8. Capriola dactylon.
⚘ 9. Cenchrus incertus.
● 10. Chamaesyce buxifolia.
⬤ 11. Chamaesyce hypericifolia.
ı 12. Cyperus brunneus.
♠ 13. Glottidium vescarium
ꝏ 14. Guilandina crista.
■ 15. Hymenocallis caymanensis.
Ӽ 16. Ipomoea pes-caprae.
Ж 17. Iva imbricata.
◘ 18. Lepidium virginicum.
ℛ 19. Leptilon canadense.
❚ 20. Melanthera brevifolia.
O 21. Opuntia dillenii.
▌ 22. Paspalum caespitosum.
Ω 23. Portulaca oleracea.
⊥ 24. Ricinus communis.
♆ 25. Rhizophora mangle.
Ↄ 26. Scaevola plumieri.
▲ 27. Sesuvium portulacastrum.
Ɔ+ 28. Sonchus oleraceus.
℘ 29. Sporobolus virginicus.
☨ 30. Suriana maritima.
▙ 31. Syntherisma marginatum.
V 32. Thespesia populnea.
T 33. Tournefortia gnaphalodes.
✖ 34. Uniola paniculata.
Λ 35. Valerianoides jamaicense.

A glance at the map shows that the island on the northeast side projects beyond the walls of the fort by a short arm, with the ruins of a series of coal-sheds and loading-trestles on its eastern side.

The southern side has quite a large extension outside the walls and on it are likewise ruined coal-sheds, a covered dock, and several old sheds, formerly pumping-houses, etc.; this portion also has the larger number of species. This is probably due to the fact that it is larger than the northern projection and hence had a larger number of buildings and people on it, and also to the fact that the dock is on this southern side and all persons and commodities entering the fort passed over this strip of land to the sally-port.

The northern strip or arm of the island has a well-mixed assemblage of species, in which *Tournefortia*, *Ipomœa*, and *Canavalia* predominate, with some *Uniola*, *Scævola*, and *Iva*. Over a tangled mass of iron rods and girders, the ruins of a loading-crane at the coal sheds, there is a luxuriant growth of *Calonyction* and several stout young bushes of *Guilandina crista*. Scattered along the western side, near the moat, are numerous *Leptilon canadense* plants.

On the eastern side of the fort, outside of the east coal-sheds on the narrow strip of soil between the moat and the beach, there was an extensive area of *Glottidium vescarium* plants, very luxuriant and of stout, tall growth. Just at the southwest corner of these eastern coal-sheds are two well-grown *Rhizophora mangle* trees, about 2 meters tall, which had flowers and fruits in 1916. On this strip also occur *Sporobolus*, *Scævola*, *Suriana*, *Chamæsyce*, *Iva*, and a few plants of *Calonyction*.

As the southeast corner of the fort is approached, the increase in species is apparent as one gets nearer the walk leading to the sally-port. In addition to the above species, masses of *Alternanthera maritima* and *Sesuvium* are seen, together with large quantities of the rather showy blue-flowered *Valerianoides jamaicense* and the long, shining, dark-green, blade-like leaves of *Hymenocallis*. Scattered with these are *Lepidium*, *Argemone*, and *Portulaca*.

Most of the species lie to the left of the walk on going in to the sally-port. Here are numerous grasses, *Paspalum*, *Syntherisma*, *Capriola*, as well as *Uniola* and *Cenchrus*, which were likewise noted along the eastern side. *Sporobolus*, also, is fairly abundant and some of the sedge *Cyperus brunneus*. Near the old tumbled-in cistern are masses of *Calonyction*, *Hymenocallis*, *Ipomœa*, and the castor-oil plant, *Ricinus communis*, resembling trees, some being 10 to 15 feet tall, and with these many plants of *Leptilon*, *Ipomœa*, and *Iva*. Along the curving shore between the dock and the southern coal-shed were several young trees of *Thespesia* and *Suriana*. These are probably self-sown in the rather sheltered nook. Along the inner side of the coal-sheds among ballast were many plants of *Sonchus oleraceus* and *Leptilon*, which are in reality ballast plants. Stretching along the ridge of sand on the western side of the southern projection is a thick association of *Uniola* interspersed with *Leptilon*, *Cenchrus*, *Iva*, and *Canavalia*.

On the inner side, near the moat, is a mixture of *Bidens leucantha*, *Melanthera brevifolia*, *Boerhaavia viscosa*, *Scævola*, and *Chamæsyce*. On the westernmost side of this area was found the only station for *Chamæsyce hypericifolia* in the entire Tortugas. These low-tufted, grayish plants grow thickly under several *Tournefortia* bushes.

Southwest of the southern angle of the fort are several large patches of *Opuntia* and a single *Avicennia nitida* tree. This black mangrove is the sole representative of the species on the island. Lansing mentions 30 of these trees having been planted inside the fort, but these must have perished, for not a vestige of them remains. This tree, which is about 2.5 meters tall, is probably a seedling drifted in by the waves or from seed carried in ballast from Key West. On the southern shore, near the dock, are numerous small patches of *Atriplex cristata.*

The interior of the fort has a large parade-ground (plate 4) and various buildings and ruins. This parade-ground has been planted with some introduced trees, as mentioned by Millspaugh, but a curious omission in that survey was the grove of large and old white buttonwood trees just within the sally-port. This grove is quite large and thick, and numerous young seedlings have sprung up among the older trees. While the fort was a military fortress, then during its period of use as a federal prison, later as a quarantine station and coaling-depot, and now under the jurisdiction of the naval authorities, up to date the order has been handed along that these old trees (the only remnant of the traditional forest or thickets which covered these islands) shall be untouched (see plate 4).

Notwithstanding the tendency and inclination of various care-takers of the fort to cut down and destroy all plants springing up inside the fort and the practice of burning over the parade-ground at regular intervals, and the serious fires in the fort which destroyed the large barracks and the keeper's cottage, these old trees have persisted to the present and are now strong and healthy. An interesting sport was noted in the grove, viz, a few young plants of *Conocarpus erectus* var. *sericius.* This variety probably arose quite recently as a genetical variation, very likely a mutation, as these gray tomentose trees are all quite young and have started as seedlings right in the midst of the old trees, which all have dark-green and glabrous foliage. The center of the old parade-ground is occupied every year by a thick stand of the weed *Glottidium vescarium,* which the care-taker tries to destroy by burning over the ground annually, but to no avail.

Of the trees mentioned, there are a few specimens of *Terminalia* and a tamarind (plate 5) which shows beautifully the direction of the prevailing wind by the one-sided development of the tree after it had grown so tall as to get the effect of the winds sweeping over the parapets. Here also about the buildings are several *Phœnix* palms, both *P. dactylifera* (see plate 5) and *P. canariensis,* a number of coconuts, some sea-grape trees, *Cocolobis uvifera* (which are quite old), a number of *Thespesia* trees, gum-elemi trees, *Elaphrium simaruba,* as well as *Cordia* and *Oleander* bushes. On the west side are several clumps of *Agave decipiens* (see plate 6) and in a ruined powder magazine there has sprung up, with a thicket of gum-elemi trees, a fair-sized guava tree, which produces fruit quite abundantly, as do also the date palms and tamarind. The parade-ground is carpeted with many small native weeds, as *Boerhaavia*, *Bidens*, *Ipomœa*, etc., as well as introduced ones, *Portulaca*, *Argemone*, *Lepidium*, *Ricinus*, *Dolicholus parviflorus*, *Cajan cajan*, *Sida procumbens* and *S. carpinifolia*, *Phyla nodiflora*, *Sonchus*, *Leptilon*, etc.

On the earth-covered ramparts built on the top of the casemates a number of plants have secured an elevated and very dry footing. These are *Opuntia*, *Melanthera*, *Canavalia*, *Valerianoides*, *Sporobolus*, *Chamæsyce*, *Paspalum*, and *Bidens*, and in shielded crevices several good-sized trees of *Elaphrium simaruba* have managed to thrive (see plates 4 and 6).

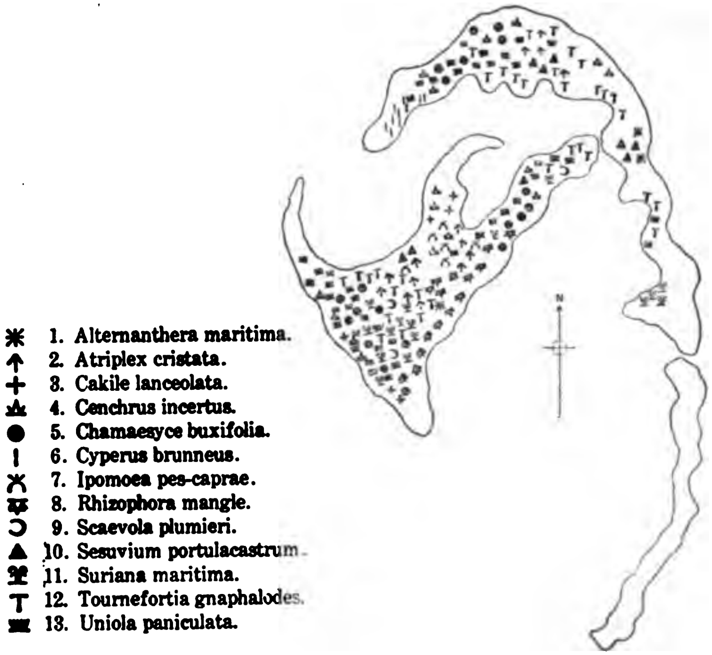
The species listed on this interesting old key are 35, the number that Lansing and Millspaugh give for the same area—*i. e.*, for the portions outside of the fort; the parade-ground is omitted as having been too much under artificial influence to be of much importance in an ecological study; but these 35 species are not identical, for 9 of the Lansing species were not obtained in this area by the author, viz, *Amaranthus viridis*, *Cenchrus echinatus*, *Euphorbia havanensis* and *E. adenoptera*, *Eustachys petræa*, *Heliotropum curassavicum*, *Sida carpinifolia* and *S. diffusa*, and *Capraria saxifragifolia*, although the last three or closely related species were found inside the fort. The 9 new species introduced into the given area are *Lepidium*, *Guilandina*, *Ricinus*, *Chamæsyce hypericifolia*, *Thespesia*, *Rhizophora*, *Calonyction*, *Avicennia*, and *Leptilon*. In cases of doubt concerning species, *e. g.*, *Sida diffusa* of Millspaugh and *Sida procumbens* S. Watson, as mentioned in this paper, the synonymy could not be exactly traced, since Millspaugh did not give the authors of his species.

BUSH AND LONG KEYS.

The general map of the Tortugas shows that these keys lie slightly west of Garden Key; they are very irregular and are merely narrow strips of sand and ridges of broken corals. During the winter season of 1911–12 the two keys were united by land connections due to storms, so that the outline is now that shown in the distributional map given here. The western end seems to have been the longest elevated out of the sea and supports the most vegetation. The eastern end, however, has tc bear the brunt of the storms and the waves driven in by the prevailing winds, and it is perhaps for this reason that the vegetation is more scant.

Lansing and Millspaugh omitted treating this key as being "so low as to be awash during heavy weather and on this account void of vegetation." If these conditions obtained then the keys must have been built up within a year or two after Lansing's survey, since stem measurements on some of the *Suriana* and *Tournefortia* bushes on this key show them to be almost 12 years old by comparison with plants of the same species from other keys. At any rate, the flora of the island is quite varied and well scattered. Singular as it may seem, it is on this island that more young plants of the red mangrove were found than on any other island in the Tortugas. The south shore has many small seedlings and in a landlocked tidal pool there is quite a clump of older mangroves, which are perhaps 3 or 4 years old and seem to be in a flourishing condition (see plate 6). This pool also contains a considerable school of small fish and some crabs.

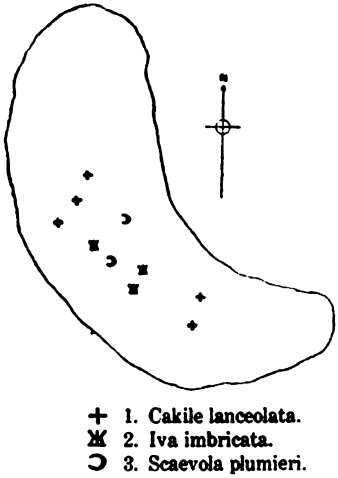
This key is the main nesting-place in the Tortugas for that beautiful and graceful little sea bird, the least tern. All along the beach near the young mangrove plants one stumbles suddenly upon the pairs of speckled eggs, which are very difficult to see as they lie on the bare coral sand. Later the downy chicks are just as difficult to perceive, for at the approach of a stranger they instantly become motionless balls of gray fluff. These least terns are the only birds nesting on the island.



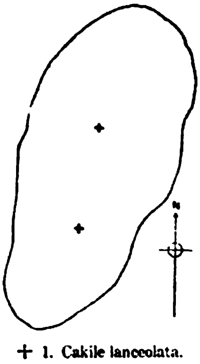
Bush and Long Keys. Two inches = one-fifth statute mile.

The western shore terminates in a long and narrow northern and a broader and more rounded southern arm. *Cakile* and *Cenchrus incertus* grow along the beach at scattered intervals. Back of this and stretching east to the tidal pool is a thick aggregation of *Uniola*, *Tournefortia*, *Iva*, and a little *Suriana*. East of the pool is a sparse growth of *Atriplex*, *Sesuvium*, *Ipomœa*, *Cakile*, and *Alternanthera*. South of the pool and in a line with the highest tide-levels are the young *Rhizophora* seedlings. These seedlings and those about the pool were sufficient to furnish the author with material for physiological work during two seasons at the laboratory and a large number are still left. From the middle of this island to the narrow tidal gut separating the eastern from the western island there is again a mixture of *Uniola, Tournefortia, Cenchrus, Atriplex, Sesuvium*, and a little *Scævola*. North of the portion across the inlet there is an association similar to this latter group, with the addition of *Cyperus brunneus* and *Chamæsyce* in large amount.

Following the curve of the western island as it bends south, the vegetation as plotted on the map is very sparse. It is this shore which gets the full sweep of the wind and waves from the northwest. The surface here is mostly heaps of coarsely broken corals and shells, and the plants it supports are a few *Sesuvium* clumps, *Suriana* and *Tournefortia* bushes, and *Cakile*. The *Suriana* is seen only on the leeward side in the more sheltered coves of the shore. In concluding the observation on these islands one can only state, in the light of Lansing's survey, which records them as barely emerged from the sea, that all the above vegetation must have appeared on them in the 12 years that have passed since 1904.



Sand or Hospital Key. Two inches = one-fifth statute mile.



Middle Key. Two inches = one-fifth statute mile.

SAND OR HOSPITAL KEY.

Stretching eastward from Garden Key and separated from each other by almost equal distances are Sand, Middle, and East Keys. All three are small and only the easternmost one, East Key, supports any real covering of vegetation. Sand Key, which is also said to have been used for hospital purposes during the yellow-fever plague of the late sixties, is only about 90 feet long and half as wide, and consists entirely of blistering and glaring white coral sand. It was visited at various times by the author during 1915 and 1916, and at the first visit he noted near the central portion of the key one plant of *Scævola* and two plants of *Iva imbricata.* These seemed to be growing and flourishing. During July, in 1915, several visits were made and on the last occasion only one dead *Scævola* plant and several dead *Cakile* plants were found. There was nothing alive on the island but a few hermit crabs and some sea birds fishing near the beach at the western tip of the island. The sand was so hot at mid-day that, after walking over the island a few minutes, shoes had to be removed and the circuit of the island completed by wading. With this incident as an illustration, it is seen that only the hardiest plants can live on these blistering sand islands.

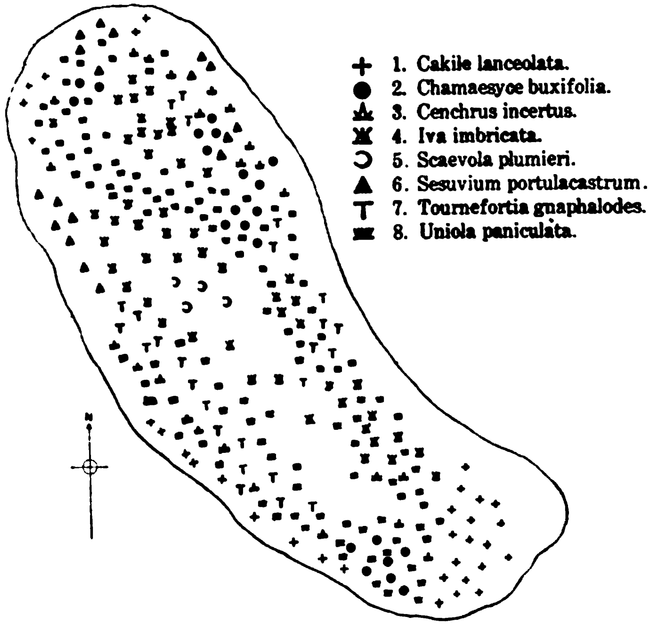
In Lansing and Millspaugh's paper, Lansing noted 5 species, fairly well scattered over the island—*Uniola* in the north, *Sesuvium* in three groups on the east, and *Iva* along the west shore, the central area being filled with *Ipomœa* and *Chamæsyce.* Since March 21, 1904, then, the whole island must have been denuded of vegetation and the three species noted by the author have come in lately. All the plants have gradually succumbed to the hard conditions on the island. Of Lansing's 5 species, only *Iva imbricata* was found by the author; *Scævola* and *Cakile* were not reported by him as being there at that time.

MIDDLE KEY.

Next to Sand Key, going east, is Middle Key, the smallest of the Dry Tortugas islands. It is little more than an oval patch of sand, about 80 feet long by 50 feet wide. Millspaugh states that Lansing found no vegetation on Middle Key whatever and that it is so low that, like Long Key, it is awash in rough weather. Notwithstanding the possibility of the last statement, the author found several large tufts of dead *Cakile* on the key, its season having passed in July. It is reasonable to suppose that the island has been built a little higher in the past 12 years by the current action, and especially so since the general map accompanying the paper shows that Middle Key is built, as are indeed the others, on quite extensive shoals scarcely submerged.

EAST KEY.

This is the most outlying key of the group since the disappearance of North and Northeast Keys a few years ago. It is the largest of the three keys stretching northeast of Garden Key and is about 4.5 miles from the latter key, about one-third of a mile in length and less than half that in width. It is almost entirely covered with vegetation and shows several associations as outlined in this paper. *Cenchrus* and *Cakile* are disseminated thickly along the shores, the latter particularly along the southeast shelf of dry sand-beach. *Uniola* and *Chamæsyce* are in fairly thick growth on the northern and southern ends, with a sprinkling of *Uniola* all along the western side. *Tournefortia*, as large, well-grown bushes, occurs on both the east and west sides of the island, but back some distance from the shore. The northern half of the key is implanted rather thickly by two patches of *Iva imbricata*, separated by a band of *Uniola*. Near the middle of the island is a single small group of *Scævola* plants. *Sesuvium* occurs on the north and northwest shores. In a comparison with Lansing and Millspaugh's survey it is seen that the plants listed are exactly the same as those in the present survey; 8 species are given, and these are the same with the exception of *Cakile fusiformis*, which is perhaps the same systematic error noted before, *C. lanceolata* being the one noted on the island at present. Taking these facts as evidence, one may assume that in the 12 years gone by all the species have held their footing and some have increased in amount, but not at the expense of the others, and as this key is very rarely visited, the ecologic influences governing the distribution of the species have been undisturbed.



East Key. Two inches = one-fifth statute mile.

SPECIAL ECOLOGY.

Seasonal differences.—As might be expected from the data presented under "climate," the conditions are fairly uniform. The general appearance of the islands is the same at all seasons of the year. Of course during the winter months and early spring, after the heavy rains in September to December, there is more green carpeting in dry places, due to various grasses and low herbs which turn brown and dry in the later and hotter summer months, and such tropical trees as the large *Ficus hispida*, planted in the courtyard at the light-house on Loggerhead Key, drop their leaves during the coolest months, December and January. But this dormant condition lasts only a few weeks; other plants, as the *Hibiscus* and oleanders, seem to bloom and flourish throughout the year, and during the summer months particularly are covered with showy crimson, scarlet, and pink blossoms.

Flowering and fruiting.—These phenomena are not definitely limited in the Tortugas region. Coconuts frequently bloom and ripen at the same period, and the same is true of the geiger-trees, some of which have showy clusters of scarlet flowers and bear on the same branch the hard, pointed, white fruits. The *Scævola* often bears its white blossoms simultaneously with the large edible-looking but nauseating black berries. Some periodicity, however, has been noted in the red mangrove and the sea-grape, *Cocolobis uvifera*. Trees were seen with blossoms and ripe or nearly ripe fruits, but never trees with blossoms together with very immature or half-ripe fruits and fully ripened ones. There must be some definite cycle of growth which prevents the occurrence of fruits at all stages in these trees.

Herbs, such as *Melanthera*, *Valerianoides*, and *Alternanthera* have buds, blooming inflorescences, and old dead and dry flower-clusters all on the same plant; other herbs, such as *Cakile* and some of the grasses, have a well-defined growing-season. *Cakile* grows only during the winter and spring; by June the plants are full of ripe seeds, and during July the plants die and become tumble-weeds. *Tournefortia* and *Suriana* seem to have cycles of growth not separated by long periods, for some individuals are frequently seen having flowers and ripe seeds. The Convolvulaceæ, represented by *Ipomœa* and *Calonyction*, appear to have all stages of flowers and fruits, for even long periods of drought do not seem to prevent *Ipomœa pes-capræ* from producing its showy rose-purple funnels along the runners trailing 20 to 30 feet across the burning white sand-beaches.

Influence of soil.—There is not much field for observation under this head, since the islands are essentially all alike geologically—*i. e.*, the soil is all white calcareous sand, but at several places in the Tortugas there is a slight variation in the soil. On Bush Key, near the small tidal pool, the soil is lower than the adjacent sand and various unicellular and a few filamentous algæ (*e. g.*, *Lyngbya*), etc., grow among the mangrove seedlings. In similar situations on Boca Grande the writer observed *Sesuvium*, *Alternanthera*, and *Batis maritima* in salt meadows or marshes where young rhizophoras were also establishing themselves. However, on Bush Key only an algal flora was observed on the surface of the soil and on the mangrove stems.

The other variations in soil were due to artificial conditions. On Loggerhead several carloads of soil had been brought from Maplewood, New Jersey, and this supports a few introduced weeds, such as

Syntherisma and *Cenchrus echinatus*, with several *Poinsettias*. On Garden Key portions of the parade-ground, possibly through long years of cultivation and some attempts at fertilization and artificial watering, show a difference in the soil from that of the rest of the key. The accumulation of some humus here also makes a change in the soil's character. This portion of the parade-ground is thickly covered with a mat of *Lippia*, various examples of *Sida*, *Dolicholus*, etc., plants which need slightly more nutriment in their substratum than is afforded by the coarse, limy gravel and sand of other parts of the key.

Human and animal influence on vegetation.—Man's influence on the plants in the Tortugas may be designated as constructive and destructive. The introduction of plants for use or ornament on Loggerhead and Garden Keys and their introduction unwittingly with ballast, etc. (such as *Leptilon*, *Sonchus*, *Syntherisma*, *Portulaca*), together with coconuts, papaws, *Casuarinas*, and *Salvia serotina* (used in earlier years as a febrifuge), may all be classed as constructive influences. The cutting down of the white buttonwood trees by the old fishermen, according to tradition, the burning off of the *Suriana* on Loggerhead, and the burning over of the parade-ground in the fort on Garden Key are destructive activities. On Bird Key the presence of the warden does not seem to have produced any change in the vegetation, while the terns which breed on the island have very little effect, as they do not feed on any plant substances and it is only the noddy terns which make nests of a few dry sticks; the other two species of terns lay their eggs on the bare sand.

Millspaugh ascribes to birds a large influence in the distribution of plants in this region, but in the writer's opinion this has been overestimated; the only birds in the region are the terns and a few frigate-birds which prey on the terns. None of these birds are waders, nor do they spend much time on land, according to the author's observation. Millspaugh mentions 8 plant species as avevectant by the feet of sea birds, six of these occurring in the Tortugas, but as there are few birds in these islands and those are of such habits as scarcely to permit of carrying seeds in the webbed-toe expansion, the hypothesis is hardly tenable. A more reasonable factor for distribution in the Tortugas is the sea alone. The undoubtedly aquavectant plants (as *Cakile*) appear on the islands no sooner than such supposedly bird-borne plants as *Cenchrus* and *Scævola*.

Other animals associated with plants are the red land-crab, *Gecarcinus lateralis*, which lives on various islands, but was observed especially on Loggerhead, where it fed on decaying leaf-mold accumulating in the grove of *Sebesten* trees, but it probably has no effect on this tree's economy. A small black beetle also lives in this tree's deep salver-form corolla and perhaps aids in fertilization. Another insect is the tiny butterfly *Thecla*, which lives on the nectar of *Melanthera* and incidentally pollinates its stigmas.

Storms.—Hurricanes and great waves driven by continuous winds have wrought more havoc on the vegetation of the Tortugas than perhaps any other agents. Whole keys have been washed away by some of the great storms, and islands have been completely denuded of vegetation. Not only on these sand islands, but in the mangrove swamps of larger islands (as at Boca Grande), large areas of swamp were dead and piled up densely with the decaying branches of shattered *Rhizophora* trees. To storms also may be laid some of the destruction of the *Suriana* on Loggerhead and Bird Keys by driving in the salt spray, which kills the bushes, or by the mechanical injury of undermining the roots by high waves and later leaving the absorptive system exposed to the drying air. This latter case is observed on the eastern shore of Loggerhead, where the bank was eroded and long ridges of the *Suriana* were dead, with their roots sticking up in the air.

Structure in relation to environment.—This subject is well illustrated in the Tortugas. All the plants of these dry islands, in which there are no springs of fresh water of any sort and where the substratum is of a loose, porous nature, must be adapted to conserve moisture. The structures facilitating conservation of moisture in the Tortugas flora are various. In *Tournefortia* and *Suriana* the leaves are densely covered with tomentum and pubescence; they are spatulate in shape, which permits their being set closely together, and as a further reaction they assume an appressed hyponastic position during the middle portion of the day or in prolonged droughts. *Scævola* and *Ipomæa*, both of which have large leaves, are heavily cutinized and frequently have sunken stomata; they also assume the hyponastic appressed position in unfavorable conditions and seasons. *Chamæsyce* has small, reduced leaves, heavily cutinized, appressed, sessile, and flap-like, which transpire very slowly. *Iva* has glabrous, heavily cutinized leaves with thick, fleshy mesophyll, containing water-storage tissue. *Cenchrus*, *Cyperus*, and *Uniola* have narrow, reduced leaves with small stomata and, together with many grasses, roll inward during dry seasons or in dry situations, the reaction being due to the thin-walled water-storage cells in the sinuses of the delicate ridges losing water, the lowered turgescence causing the leaf to involute.

A peculiar reaction to environment was noted in the *Poinsettias.* Individuals of species occurring in the Tortugas were found in more favorable situations on the mainland of Florida with rather broad blades; with variations in environment and decreasing moisture and shade, all stages were seen, down to leaves which were little more than midribs. *Boerhaavia* has a rather broad blade, but is protected from excessive transpiration by tomentum and glandular hairs. Thus it is seen that on minute examination nearly every plant in this region has some special protective feature to guard against loss of water, the acme being reached, of course, in *Opuntia,* which has no leaves whatever, a thickly cutinized epidermis, and mucilaginous sap.

In this connection the actual transpiration records taken by the writer with a potometer in many of the representative plants of the Tortugas prove very illuminating. The records were taken in both sun and shade and are given in condensed form in the table following:

Potometer transpiration-rate averages.

No.	Species.	Weight.	Quantity.	Length of time in sun.	Length of time in shade.
		gm.	*c.c.*	*h m*	*h m*
1	Suriana.......	.6	1	1 15	1
2	Chamæsyce...	3.7	1	1 50	2 10
3	Canavalia....	9.1	1	22	40
4	Tournefortia..	3.5	1	10	12
5	Ipomœa......	3.6	1	1 25	37
6	Boerhaavia...	4.5	1	36	25
7	Scævola......	7.5	1	13	25

It is seen that some plants have transpired the given quantity of 1 c.c. in a shorter time in shade than in sun. These are *Suriana*, *Ipomœa*, and *Boerhaavia*. This apparent contradiction of the law of transpiration, which expresses the fact that the higher the temperature the greater the water transpiration, is explained by the fact that in the intensely hot sunlight and the reflection and brilliant glare of the white sand those plants were heated beyond the point at which transpiration was at a maximum and the leaves lost so much turgescence that they wilted and became flaccid, with an almost complete inhibition of transpiration. This phenomenon is observed more particularly in young tissue, as tender shoots and branches which still have collenchyma in the stems and poorly developed epidermis on the young leaves. It was necessary to use such shoots of the three above-mentioned plants, since the older and more woody stems could not be fitted into the small Ganong potometer. However, this reaction was noted in plants growing *in situ* just as well as in the potometer subjects.

In several patches of *Boerhaavia* along a walk at the laboratory it was noted that during hot days, from before noon to mid-afternoon, the younger shoots would be hanging limp and flaccid, while about 4 p. m. they would assume their wonted erectness with turgescent leaves. Plants in more northern climates also frequently show this; notably *Dahlias* planted in open sunny places have often been observed by the writer to have all the upper leaves completely wilted in hot August weather during the middle portion of the day, while with the approach of evening they completely recovered. This temporary loss of turgescence may occur daily without apparent injury to the plants.

Of all the plants tested, *Chamæsyce* seems the best protected and transpires the given quantity in the longest time. *Suriana* and *Ipomœa* come next, while *Tournefortia* with its dense silky tomentum and *Scævola* with thick fleshy leaves heavily cutinized show relatively little difference in the rates in sun or shade conditions and actually give off a cubic centimeter of water in a very short period—*i. e.*, in about 14 minutes. It is thus seen that this physiological action of loss of water varies considerably, even in these plants adapted to these xerophytic conditions and highly specialized morphologically.

MARINE ECOLOGY.

The submerged plants growing about the Tortugas are not many, since marine algæ most abound on rocky shores and rocks are conspicuously lacking in the Tortugas physiography. Of the higher plants several interesting spermatophytes are very abundant, viz:

(1) *Thalassia testudinum* Köenig and Sims, which occurs on extensive areas in shallow water. The leaves and stems of this plant, the turtle-grass, are cast up in long rows by the waves, particularly after storms, on the sandy beaches of the islands.

(2) Another component of this mass of débris is the manatee-grass, *Cymodocea manatorum* Ascherson, which has much more slender leaves and a peculiar inflorescence. It was the author's good fortune to secure both of these plants in bloom, and the *Cymodocea* in fairly large quantity by going down in a diving helmet in about 3 to 4 meters of water and walking about on the bottom, making a close observation of the beds of this grass. Algæ were also collected in the same manner.

(3) Two other spermatophytes, which were obtained by the deep-sea dredging apparatus carried by the Laboratory yacht, were two species of *Halophila*, growing in 17 to 19 fathoms of water out in the Gulf Stream. These were *H. engelmannii* and *H. baillonis* Aschers. The occurrence of these plants in this locality and their interesting relations to the region have been discussed by the writer in another paper.[1] These and the calcareous algæ which are given in a list below are quite abundant. Representatives of the Rhodophyceæ and the Phæophyceæ are not nearly so plentiful. The *Udotea* and *Penicillus* are quite common in sandy shallows and various species of *Halimeda* contribute a large share to the detritus making up the land. *Codium* and *Acetabularia* are frequent on rocks, shells, or other submerged objects affording a firm foothold. *Gracillaria* is mostly brought up on shells and sponges in the dredges along with species of *Halophila*, while *Sargassum bacciferum*, and occasionally *Valonia*, *Dictyosphæria*, *Lyngbya*, *Hypnea*, and *Lawrencia*, drift about the islands and in the Gulf as large mats or rafts. These are washed up on the shores in long windrows, and while floating these mats of *Sargassum* furnish an abiding-place for numerous small animals, especially several species of crabs. One which is fairly common matches exactly the yellow-brown fronds of the *Sargassum* and can be seen only with difficulty in a tangle of the gulfweed when thrown into an aquarium.

---

[1] Bowman, H. H. M.: Adaptability of a Sea Grass. Science, n. s., vol. XLII, 1103, pp. 244–247.

List of Submerged Spermatophytes and Algæ in the Gulf of Mexico about Tortugas.

Order NAIADALES.

Cymodocea manatorum Ascherson. Manatee-grass.

Order HYDROCHARITALES.

Family Elodeaceæ.

Halophila engelmannii Ascherson.
Halophila baillonis Ascherson.

Family Hydrocharitaceæ.

Thalassia testudinum Köenig and Sims. Turtle-grass.

ALGÆ.

Cyanophyceæ.

Lyngbya majuscula (Dillwyn) Harvey.

Chlorophyceæ.

Caulerpa sertularioides (Gmelin) Howe.
  prolifera (Förskal) Lamarck.
  racemosa (Förskal) Agardh. var. *occidentalis* (J. Agardh.) Börgesen.
  ashmeadii Harvey.
  crassifolia (Agardh.) J. Agardh.
Codium tomentosum (Hudson) Stackhouse.
Penicillus dumetosus (Lamarck) Decaisne.
Udotea flabellum (Ell. and Sol.) M. A. Howe.
  cyathiformis (Decaisne) Howe.

ALGÆ—continued.

Chlorophyceæ—continued.

Halimeda simulans M. A. Howe.
  tuna (Ellis and Solander) Lamarck
  tridens (Ellis and Solander) Lamarck.
  opuntia var. *minor* (Ellis and Solander) Lamarck.
Valonia ventricosa L.
Dictyosphæria favulosa (Agardh.) Decaisne.
Acetabularia crenulata Lamaroux.

Phæophyceæ.

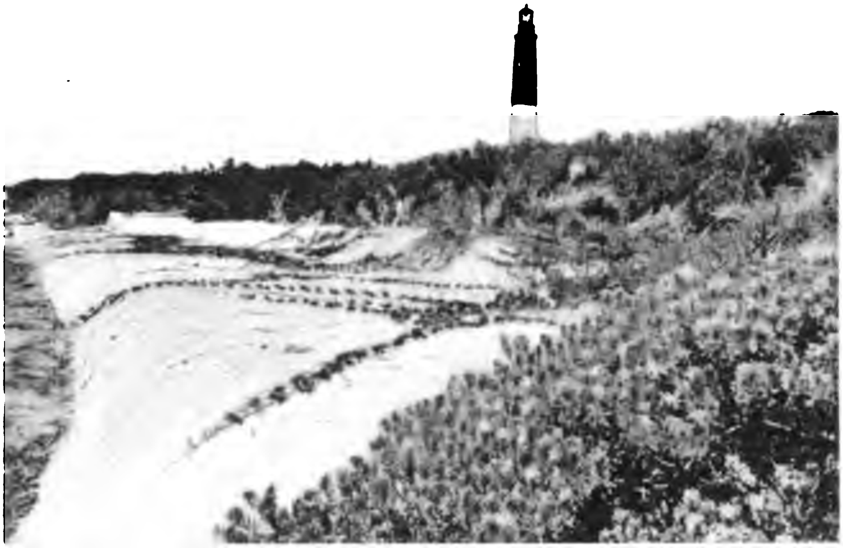
Dictyopteris justii Lamarck.
Zonaria lobata A. K. Agardh.
Padina pavonia (L.) Gaillon.
Sargassum natans (L.) Meyer—*S. bacciferum*

Rhodophyceæ.

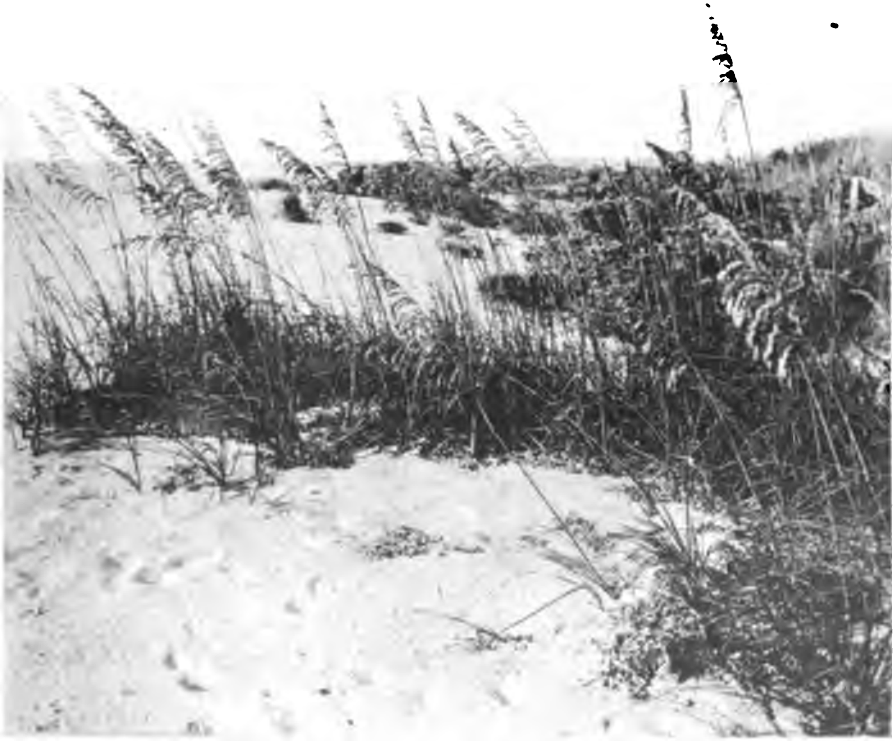
Hypnea muscoformis (Wulfen) Lamarck.
Acanthophora spicifera Vahl, *A. thierii* Lamarck.
Lawrencia obtusa (Hudson) Lamarck.
Galaxaura flagelliformis Kjellman.
Wurdemannia setacea Harvey.
Corallina rubens L.
Gracillaria confervoides (L.) Greville.

CONCLUSION.

In this treatment of the species in the Tortugas it has been aimed to give some idea of the character of the dry-climate plants inhabiting these islands, their distribution, and particularly the changes which have occurred on the various keys since Lansing's survey, with an attempt to analyze the reasons for such changes. This, it is hoped, has been done, with the help of herbarium specimens collected and living plants sent north to the University of Pennsylvania Botanical Garden, and also the extensive field-notes and laboratory experiments made by the writer during his residence of two summers in these islands.

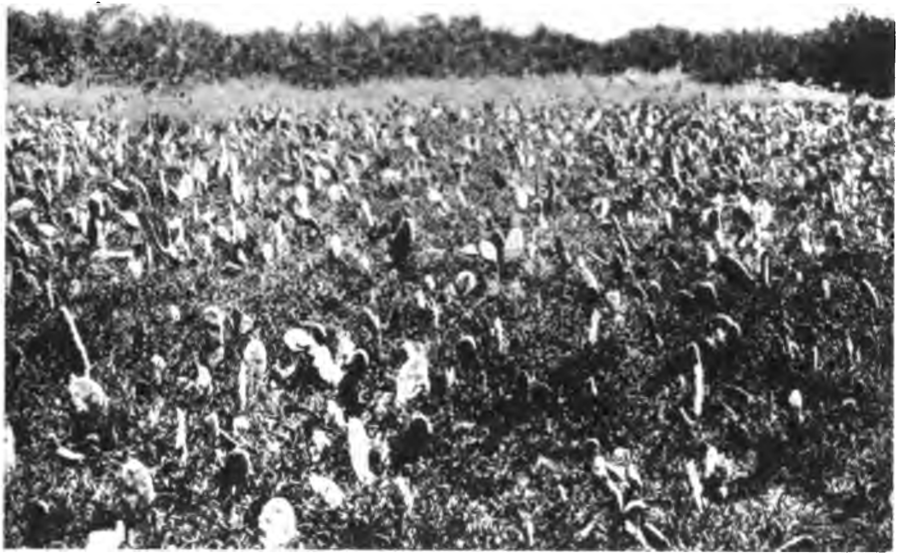


1

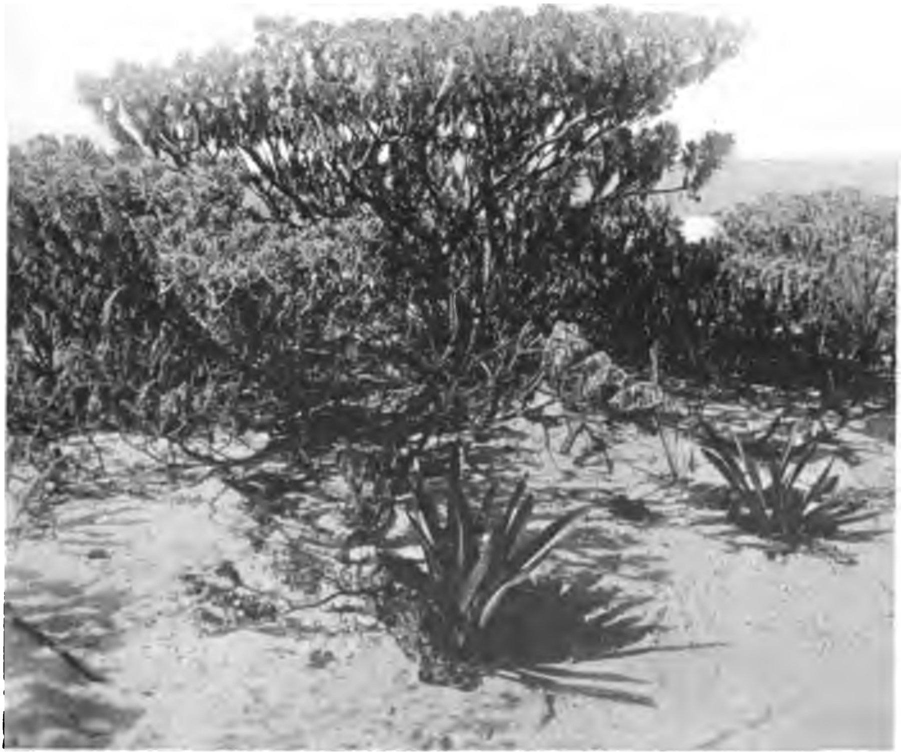


2

1. Beach terraces on Loggerhead Key showing *Suriana maritima* in background, runners of *Ipomoea pes-caprae* and *Tournefortia gnaphalodes* in foreground.
2. Beach on Loggerhead Key showing *Uniola paniculata*.

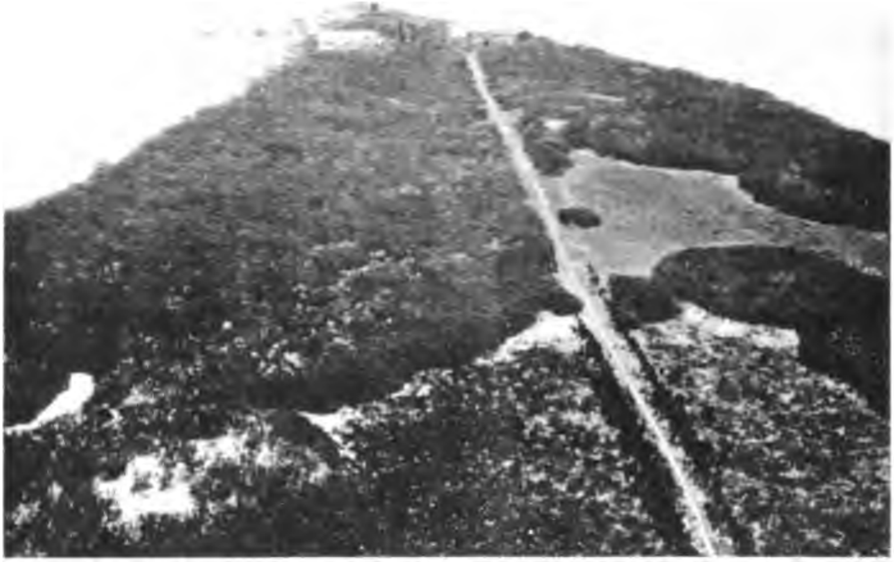


3

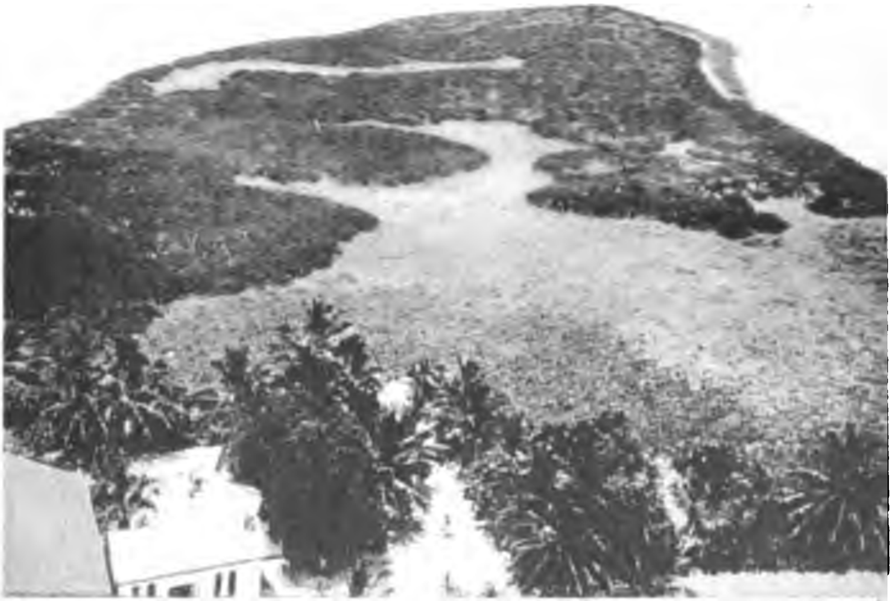


4

3. Large area on Loggerhead occupied mostly by *Opuntia dillenii*.
4. Pure stand of *Tournefortia gnaphalodes* with several plants of *Hymenocallis caymanensis* in the foreground.



5

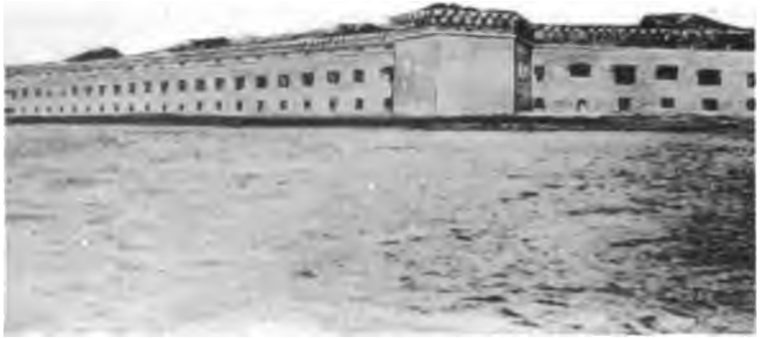


6

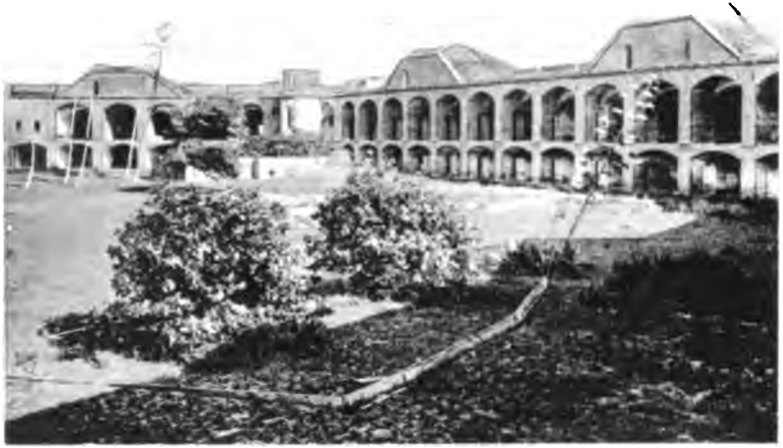
5. View of Loggerhead from the top of the Lighthouse looking north-east. Dark areas occupied by *Suriana* and lighter ones by *Opuntia* and *Calonyction*. Laboratory buildings near extreme point.

6. View of Loggerhead from top of Lighthouse looking south-west. Dark areas *Suriana* and lighter ones *Opuntia*, *Ipomoea*, *Paspalum*, *Cyperus*, *etc*. Cocoanut palms in lighthouse enclosure in foreground.

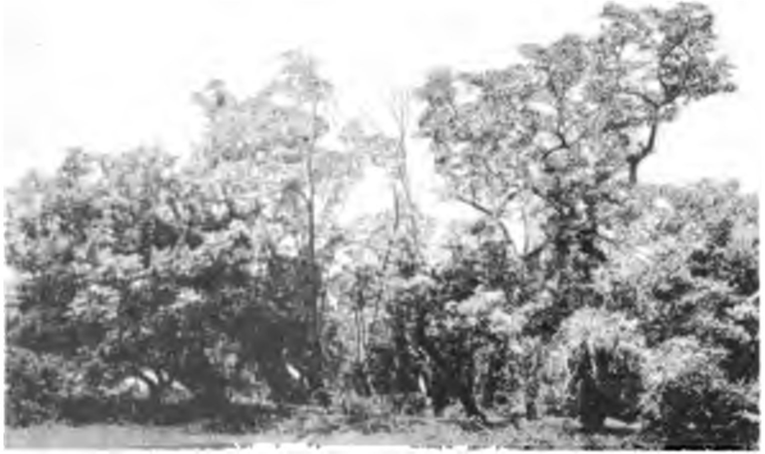
7



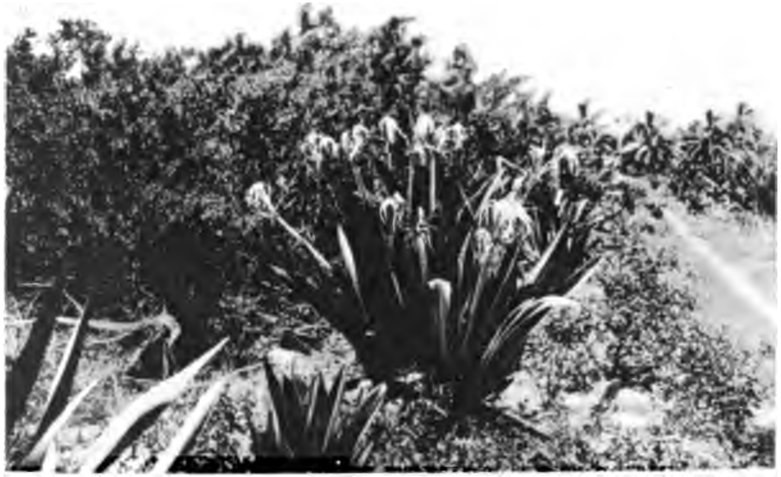
8



9

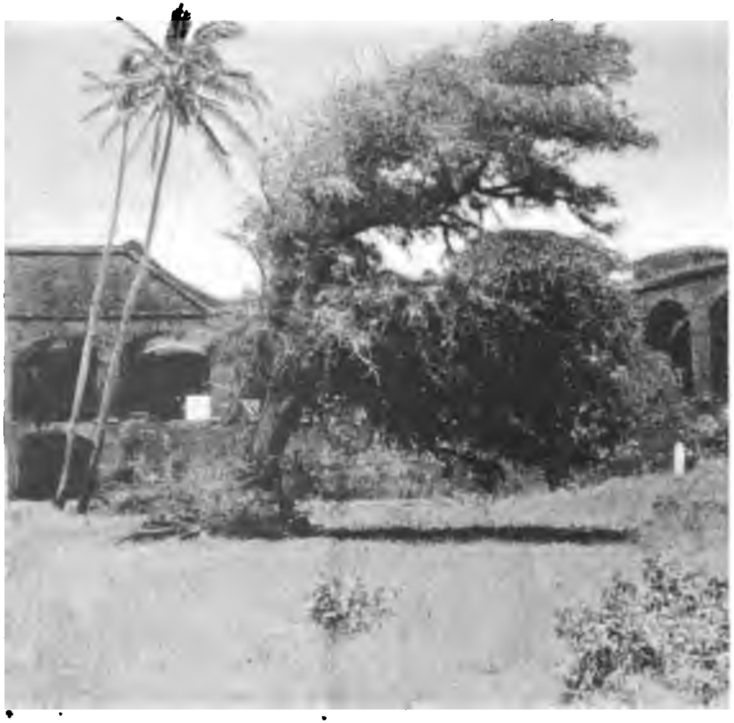


10

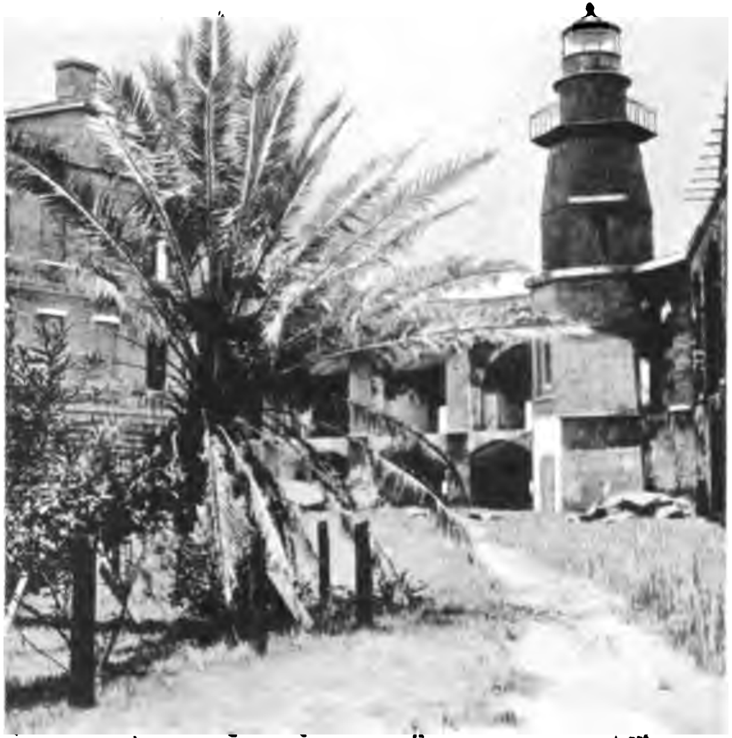


7. Old Fort Jefferson on Garden Key. Vegetation is shown growing high up on the earth-covered ramparts and bastions.
8. View of Parade Ground in Fort Jefferson showing *Sebesten* bushes. Inflorescence of *Agave,* Guavas and Gum-Elemi bushes in the background.
9. Grove of ancient *Conocarpus* trees in the Parade Ground at Fort Jefferson, a remnant of the traditional, original, silvan flora of the Tortugas.
10. Plant of *Hymenocallis caymanensis* in bloom.

11

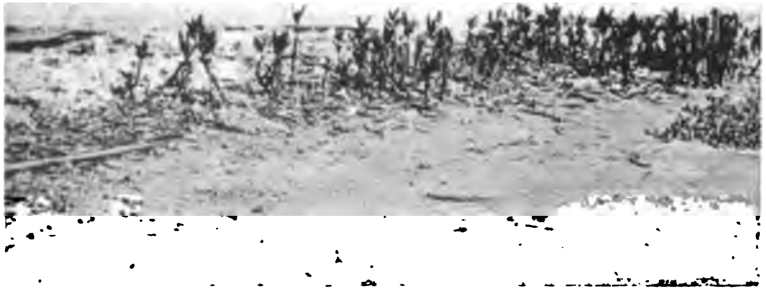


12

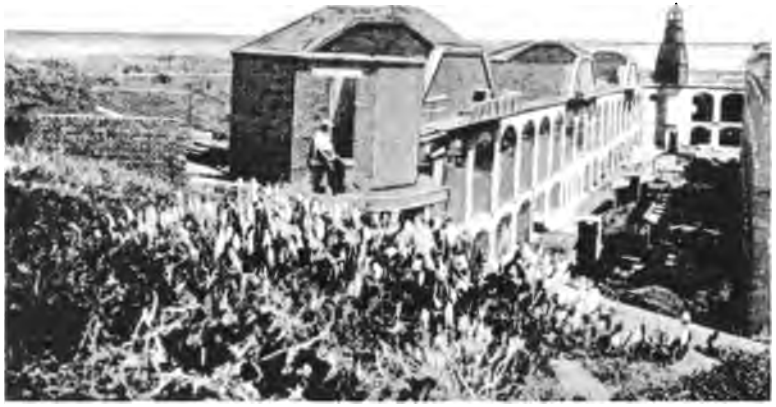


11. Coconuts and old Tamarind Tree in Parade Ground illustrating the direction of the prevailing wind.
12. Oleanders and *Phoenix dactylifera* bearing clusters of dates in Parade Ground at Fort Jefferson.

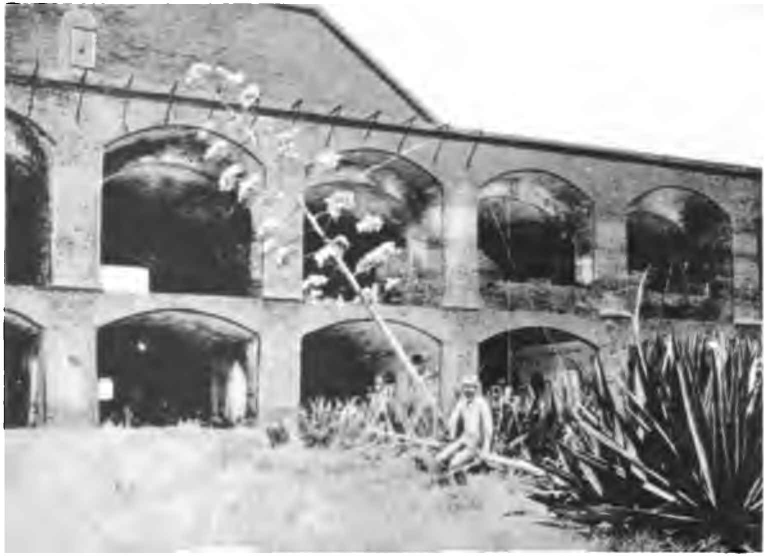
13



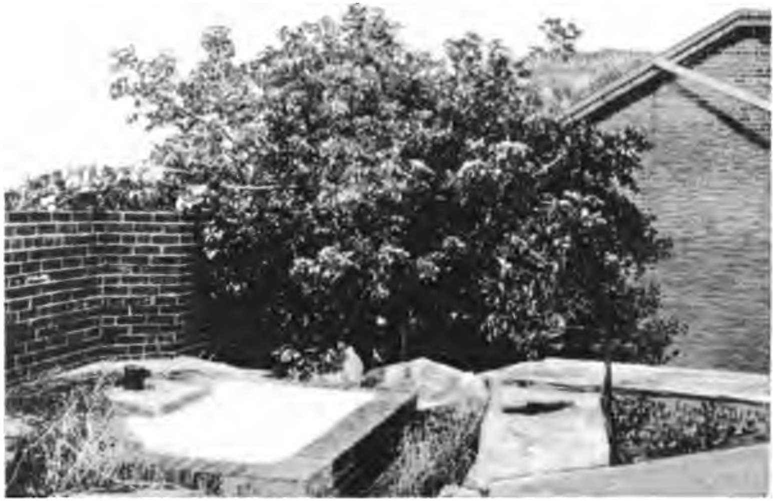
14



15



16



13. Young mangrove seedlings on the beach at Long and Bush Keys.
14. *Opuntia dillenii* growing high on the ramparts at Fort Jefferson.
15. Inflorescence and plants of *Agave decipiens*.
16. Gum-Elemi Tree, *Elaphrium simaruba*, growing in a crevice of the parapet at Fort Jefferson, Garden Key.

VI.

THE ORIGIN OF THE ELECTRIC ORGANS IN ASTROSCOPUS GUTTATUS

By E. GRACE WHITE

Seven plates, one text-figure

THE ORIGIN OF THE ELECTRIC ORGANS IN ASTROSCOPUS GUTTATUS.

By E. Grace White.

INTRODUCTION.

Living organisms, aside from expending energy in the growth, differentiation, and reproduction of their bodies and in secreting many substances useful in the preservation of their lives, may also release energy in the form of motion, electricity, heat, and light, all of which may play a more or less important part in the maintenance of life. Of these powers, that of producing electricity specifically and in quantities large enough to be of use to the organism is confined to seven groups of fishes, two of which are elasmobranchs and five teleosts. The same power has been reported to be present in a snail and in some insects, but these reports have not been confirmed. It seems clear that among the fishes the power to produce electricity has been developed independently in the seven groups mentioned. All the activities of cells, such as secretion, motion, or nerve-conduction are accompanied by the release of a very small amount of electric energy, which apparently can not be of any possible use to the animal. Are the powerful electric discharges, sometimes over 100 volts in strength, which occur in these seven groups of fishes the result of evolutionary processes that first took their origin in the very minute electric discharge of the ordinary forms of tissue-cells?

A beginning to an answer to this question may be made by studying the structure of the electric organs, and particularly their morphological and cytological development in the ontogeny of the forms. This the writer has attempted to do, as suggested by Professor Ulric Dahlgren, of Princeton University, in the case of the remarkable electric organ of *Astroscopus guttatus* and *Astroscopus y-græcum*. I wish here to express my gratitude to Professor Dahlgren for his generous offer of the material, for his kindly direction of the problem, and for his most valuable criticisms. I wish also to thank Professor E. G. Conklin, of Princeton University, and Professor E. B. Wilson, of Columbia University, for reading and criticizing the paper. The expenses of collecting the material for this work were met by donations extending over a series of years from the Carnegie Institution of Washington, through the kindness and interest of Dr. A. G. Mayer.

HISTORICAL SURVEY OF THE SUBJECT.

GENERAL.

The power of certain fishes to give electric shocks has been known from the earliest times. A hieroglyphic representation of *Malopterurus electricus*, the electric cat-fish, has been preserved on the Egyptian tomb of Ti (Gotch 50), and the still more formidable shock of the electric ray of the Mediterranean was used by the Roman physicians as a charm to cure their patients, procuring for the fish the popular and familiar name of torpedo (Dixon 38). The numbing power of *Gymnotus* has long been feared by the South Americans, and many accounts of the extraordinary behavior of this giant eel-like fish have been published from time to time in popular form (Gotch 50). Rumor would extend this extraordinary power to the snail *Daudabardia*, and General Davis describes the "wheel-bug" of the West Indies, *Reduvius serratus*, with electric organs in its legs (Dixon 38), but these reports have not yet been substantiated.

The seven groups of fishes which have this electric power represent a wide range in structure and environment. *Torpedo* and the Rajidæ are marine elasmobranchs and are of very similar structure, being broad, flat, of slow motions, with a habit of lying on the bottom of the ocean. *Torpedo* is especially common in all warm seas, but the skate has been found much more widely dispersed in the salt water of the world. *Malopterurus*, *Gymnotus*, and the Mormyridæ are fresh-water teleosts. *Malopterurus* differs considerably from the other two groups in structure, being a silurid of considerable size. It inhabits the River Nile. *Gymnotus* is a South American eel-like fish of great length, found abundantly in the South American rivers. It has the characteristic eel-like method of locomotion. The Mormyridæ are typical teleost fishes of great variety of shape and difference of habit, abounding in the Nile and other fresh waters of Africa (Gotch 50).

Although the numbing effects of these fishes have been known so long, the cause remained a mystery until in 1773 Dr. Walsh (92) discovered the presence of "intense electrical currents" in *Torpedo*, "developed through the functional activity of special organs situated, on each side, in the lateral mass of the body of the fish." In 1835 a description of the electrical organs of both *Gymnotus* and *Torpedo* appeared in the works of J. Hunter (63), while in 1844 Stark (87) and in 1846 Robin (78) described similar organs on either side of the spinal column in the tail of *Raja*, although no shock could be felt by the hand. From that time the literature on the subject has constantly increased until now every branch has been covered. The majority of the investigations were carried on during the latter part of the nineteenth and early part of the twentieth century, but a few investigators have continued the study to the present day.

STRUCTURAL.

The general structure of the organs in *Torpedo* had been described in 1678 by Lorenzini (70), and a detailed account was published in 1835 by John Hunter (63), but the anatomy of the electric organs in general was not given much consideration by investigators until the discovery, by Bilharz, in 1857 (16), of the electrical plates in *Gymnotus*. Ballowitz worked out the fine anatomy of the electric organs of *Torpedo*, *Gymnotus*, and the Rajidæ, to the latter of which he gave the name of the "pseudoelectric fishes," and to the organs the name of the "pseudoelectric organs," inasmuch as their structure resembled that of other electric organs, while their function was not then known. Ogneff (75) and Max Schultze (85) made careful and accurate observations of the innervation of these organs and of the finer details of the nerve-endings of the same.

The peculiar structure common to all electric fishes is the semitransparent material, of the consistency of jelly, which lies between and around the electroplaxes and in which lie the connective-tissue septa and numerous nerve-branches. In *Torpedo* these are two large masses, concave on the inner border and divided by septa into hexagonal columns, the ends of which lie under the dorsal and ventral surfaces of the skin. In *Torpedo occidentalis* there are 1,969 columns, in *Torpedo ocellata* but 450 (Gotch 50).

In *Raja* there are two very slender organs, tapering at both ends and lying one on each side of the spinal column. These organs are formed from and lie embedded in the muscles of the tail.

In *Gymnotus* there are four such organs, two dorsal and two ventral, of which the dorsal are the larger. The organs are divided into columns which run variable distances from the cephalic end to the tip of the tail of the fish, which may be from 8 to 10 feet in length.

Mormyrus has four organs formed from and replacing the posterior regions of the muscles of the tail. The long diameter is parallel to the spinal column, and with the general position very like that of the organs in *Gymnotus*, though not extending so far into the anterior end of the body. In *Gymnarchus* there are eight cylindrical organs, four on each side, embedded in the muscle-tissue of the tail, as close to the median bony parts as a little connective tissue in between will permit.

The organ of *Malopterurus* is peculiarly situated in the thickened skin and is imperfectly divided into halves by a connective-tissue septum on the ventral surface. It enwraps the whole body except the fins and the head.

The finer anatomy of the electric organs differs considerably in the different groups. In *Malopterurus* there is no obvious arrangement into columns, the organ being composed of lozenge-shaped electroplaxes, dove-tailed into each other and surrounded by the characteristic jelly-like material. Each half of the organ is innervated by a single gigantic nerve-cell, with but one axis cylinder, the branches of which reach all of the somewhat over 72,000 compartments in each half of the organ and are estimated to have a peripheral distribution unsurpassed by that of any other single nerve-cell (Gotch 50).

In all the other electric fishes the organs are divided into columns, themselves subdivided at regular intervals by connective-tissue septa and fixed at their peripheral edges by the boundary wall. Each of these compartments contains a protoplasmic mass, the electroplax, in which the efferent nerves end, while the rest of the space is occupied by the transparent material. The essential elements are much the same in all. The protoplasmic mass with its supporting fibers, cross-striations, and nerve terminations is called the electric plate, electric disk, or electroplax. The nerves on coming in contact with the electroplaxes branch in a characteristic dichotomous manner in most of the groups.

In *Raja batis* a band of parallel wavy fibers crosses the transparent area, still possessing the optical properties common to ordinary striated muscle-fibers and representing the vestiges of the cross-striation of the voluntary muscle-fibers, from which, according to Ewart and Engelmann (39 and 41), the electric plate has been derived. This layer will be termed the striated layer for convenience in this paper, although the striations are no longer wholly comparable to those of muscle-tissue and although they have almost entirely disappeared in *Gymnotus* and *Torpedo*, the strongest of the electric fishes. In *Torpedo* there is a faint fibrillation in each electroplax during its early embryonic state, which is completely resorbed during development. In *Gymnotus* and in *Malopterurus* no indication of the striations is visible even in the earliest stages yet seen. It is therefore evident that the loss of definite striation is correlated with the gain in electrical power and it is possible that the evolution of the electric organs may follow the loss of the original muscle striations.

PHYSIOLOGICAL.

Perhaps the best-known of all the investigators of electric fishes is DuBois-Reymond. The founder of electro-physiology, he has exhaustively covered the subject of electrical currents in muscle, nerves, and electrical organs, carrying on, in the early eighties, a prolonged dispute with Hering (57) and Hermann (58, 59) concerning the extent of polarization arising in a muscle when an electrical current has been passed through it. DuBois-Reymond experimented on living *Malopterurus* and on living *Torpedo* in Berlin, and under his direction Sachs worked on living *Gymnotus* in South America. Their combined results are so comprehensive and so accurate as to leave room for no further investigation of the same nature, and the name of DuBois-Reymond is recognized to be that of the leader in the physiological researches on animal electricity.

Cavendish in 1780 (29) was the first to conceive of the idea of current-curves and to imitate the shock of *Torpedo* by ordinary electricity. He was far in advance of his time and it was not until a century later that Faraday (42) reached the same opinion. Although it has since been possible by modern methods and apparatus to considerably modify and correct the current-curves of Cavendish, the basic idea remains his.

In 1831 Colladon of Geneva, experimenting on the *Torpedo*, gave the name of "Colladon's currents" to those currents running between points on either the ventral or the dorsal surface (31). A few observations were made the same year by Matteuci (72), but not until the discovery by Bilharz in 1857 (16) was any further advance made in the study of the electrical currents. DuBois-Reymond then calculated that "the greater the length of a *Torpedo* column, provided the number of plates in the unit of length is the same, so much greater must be its electromotive force; and by as much as the columns diminish in height from the inner to the outer edge of the organ, by so much may the electromotive force of the median columns be greater than that of the outer ones" (18). At the same time he discussed the matter of immunity in electric fishes, finding that all electric fishes are practically immune to their own shock and relatively so to the shock of another fish of the same species, much as a viper is immune to its own poison, in spite of the fact that the body of the fish is much more favorably placed to receive the shock than are the bodies of neighboring fishes. Definite electric currents are known to pass through the body of the fish. They have been detected in the digestive tract, in the brain and spinal cord, and found to be of appreciable intensity, yet the fish remains apparently unaware of the shock.

Marey in 1879 (71) was the first to represent graphically the reflex currents of *Gymnotus* and *Torpedo* by the use of the telephone. Schönlein (83) repeated the experiments with a galvanometer and calculated the voltage of the individual electroplaxes in *Torpedo* and in *Raja;* Cremer (Gotch 50) used the "saitenelektrometer" of his own invention, and in 1899 Garten (48) made very accurate measurements and records with the capillary electrometer. In 1881 Sachs (80) published his work on the physiology of the organs of *Gymnotus;* while Gotch and Burch (52, 53) and Koike (66) have independently published similar works on *Malopterurus*, so that the shock in these three fishes has been exhaustively investigated. The general results have shown that electric organs have much the same electrical properties as muscles and nerves, with the difference that in the case of the electric organs the elements are considerably modified and so arranged in series as to give the effect of a battery in which an otherwise insignificant current can be made measurable by addition (Gotch 50).

Gotch and Burdon-Sanderson (27, 28) followed up these experiments on the weak electric fishes, finding them to be similar in both the

Rajidæ and the Mormyridæ to those of the strong electric fishes, except in the intensity of the shock. The true electrical nature of these organs, which had been previously considered electrical from their structure but had not been determined by experiment as to function, was now ascertained, for definite electrical currents could be detected by the galvanometer.

The combined results of these investigators, notwithstanding certain prolonged controversies, have led to a pretty definite agreement as to the nature of the activity of these organs. The activity of the electric organs is dependent upon the arrangement of the electrical plates in a linear series for its adequate expression, and the columnar structure of the organ thus modifies rather than determines the characteristics of the activity. The nature of these activities lies in the electrical change in the nerves themselves, expressed through the agency of the electroplaxes. That the disk is the excitable structure responding to impulses from the nerves is an abandoned theory contradicted by all experimental evidence, for such paralyzing drugs as atropin and curare, which are known to effectively destroy the action of muscle-tissue, have no effect upon the activity of the electric organs unless given in such doses as to paralyze the nerves themselves. All the effects in the electrical organs are connected with excitation, and its concomitant alteration, which is always accompanied in the nerve by rapid electric changes, while the structural nature of the organ is such that the changes in each group of nerve terminations may become conspicuous by summing with those of the neighboring groups (Gotch 50).

Another law which has been generally accepted is that of Pacini, in connection with the probable direction of the current in the different electric fishes. He states that the electric current will run from the electric layer where the nerve-endings are, to the nutritive layer, so that the electric surface will be negative to the nutritive one. Experiment has so far verified this law in every case which has been examined in this regard, except in that of *Malopterurus*, in which the current goes in the opposite direction, namely, from the nutritive to the electric layer.

EMBRYOLOGICAL.

In 1877 Babuchin (4), in 1888 Ewart (40), and in 1894 Engelmann (39) worked out the origin of the electric organs of *Torpedo* and the Mormyridæ, then known as the pseudoelectric fishes, showing that, in general, electric organs have arisen by the modification of certain striated muscle-cells. In *Torpedo* and the Rajidæ each electroplax arises from a single cell, but Babuchin has shown (2, 3) that in the Mormyridæ a syncytium of cells from the fibers of the sacrolumbalis muscles may go into the formation of one electroplax. Dahlgren has shown that each electroplax probably arises from the union of all the muscle-fibers in one myotome. It was in the midst of these researches that Babuchin (2) came to the conclusion that, inasmuch as the origin, structure, and functional activity of the pseudoelectric organs, although diminutive and less highly differentiated than those of *Torpedo* and *Gymnotus*, nevertheless are fundamentally the same; therefore, "Es existieren keine pseudoelektrischen organe, es gibt nur grosse und starke, kleine und schwache elektrische organe." The term pseudoelectrical organs has since been definitely abandoned and the Mormyridæ and the Rajidæ are known as the weak electric fishes.

In 1880 Fritsch (44) described in detail the electric organs of *Malopterurus* and startled investigators by attempting to show that the electroplaxes were neither arranged in parallel series, as in all other electric fishes, nor derived from muscle-cells. He tried to prove that the organs in this fish are developed from certain gland-cells in the skin, an observation which aroused much discussion and which never has been substantiated. No investigator has been able, on the other hand, to prove the origin of the organs from any of the muscle-cells, so the matter remains an open question to this day, with a possibility of thus bringing *Malopterurus* into line with the other electric fishes. The peculiar structure of the electroplaxes, however, and the inability to harmonize the direction of the current with Pacini's law are facts which can not be denied, and *Malopterurus* stands as the great exception to the general rules for electric fishes. At any rate, *Malopterurus* has not sacrificed any of its motor muscles to the formation of the organs and its movements are not in any way hampered, while the electric coat, which completely surrounds the body, forms an effective protection as well as a means of easily capturing its food. *Gymnotus* and *Torpedo*, on the other hand, move so slowly that they have to numb their prey at some distance and then follow them up at their leisure. It is doubtful whether the weak electric fishes use their electric organs for either protection or for capturing food, although it is possible that they may capture in this way small crustacea and other minute invertebrates.

Recently Professor Dahlgren has become interested in certain American species of electric fishes. He has published several papers, one on the anatomy and muscular origin of the electric organ of *Gymnarchus* (32), an African form distantly related to the Mormyridæ, and several on the gross anatomy and habits of the star-gazer, *Astroscopus* (33, 34, 36), a marine teleost of the toad-fish group, a group hitherto unrepresented among the electric fishes. In 1906 Dahlgren (36) and Silvester published an account of the adult *Astroscopus*. The authors had been interested in reports from Charles H. Gilbert and J. A. Henshall (Jordan 64, 65), who reported having felt shocks from the two species *Astroscopus guttatus* and *Astroscopus y-græcum*. Fishermen who were interviewed said that they had always known of the numbing power of these fishes and had often received shocks from stepping on them as they lay buried in the sand.

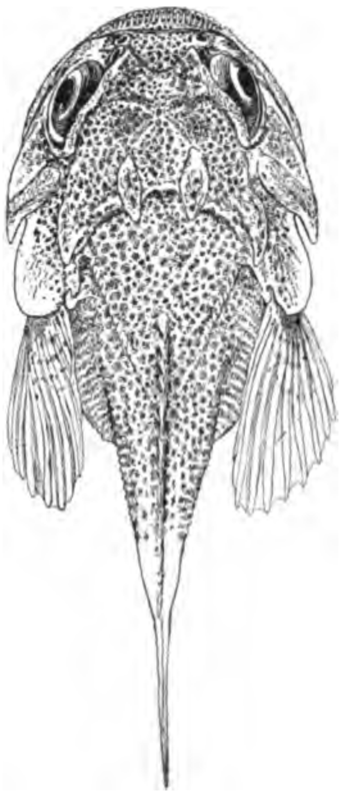
The organs of *Astroscopus* form two irregular, vertical columns, one just behind and somewhat under each eye, the muscles of which are embedded in the organ. Each organ is roughly oval in section and is composed of a number of parallel plates separated by electric connective tissue and made up of about 20 separate electroplaxes lying side by side. The number of plates in each organ does not exceed 200, but they are very thin and their constituent electroplaxes are deeply indented on the edges. The electroplax bends on itself so as to overlap on its own body at some points of considerable area, and so as to make it necessary for the overlapping portion to find room in the next layer in which to secure nerve and blood supply. From 3 to 5 of the larger electroplaxes form the central area of the layer and from 8 to 12 smaller ones are arranged around it and fill in the outline. The electroplax is placed in the organ with the electric layer upward and the nerve supply approaching it from above. The blood supply comes into contact with it on the lower or nutritive surface, which is evaginated into a number of papillæ that occupy more than two-thirds of the thickness of the electroplax. The nuclei of these papillæ are oval and differ slightly both in size and shape from those of the electric layer, which are evenly arranged in a series. The striation of this layer is remarkable and quite as definite and conspicuous as in the weakest of the Rajidæ (plate VI, fig. 3). It consists of fine, sharp lines spaced evenly and parallel to one another, but curved in many directions. In section one appears to be looking on various surfaces, the striations on one surface being parallel to each other but crossing above and below other sets of similar parallel striations. Dahlgren believes these lines to represent the edges of an equal number of curved and parallel surfaces, seen in actual or optical section. There is no variation in the distance between the lines, however, and no oblique or surface views of the striations appear (Dahlgren 36).

The nutritive layer is composed of two parts, one of which includes the nutritive nuclei, the evaginations, and the heaviest area of the striations. In the other layer there are no nuclei, but the striations continue and can be traced even into the thin electric layer, which contains a row of evenly spaced nuclei and the peculiar rods described by Dahlgren and in further detail by J. G. Hughes, of Professor Dahlgren's laboratory (62).

The nerve supply of *Astroscopus* consists of a number of medullated fibers that run between the layers in the electric tissue and end at all points on the electrical surface. The origin of the nerve supply, as well as the origin of the electric organs themselves, could not be surmised by Dahlgren and Silvester (36) at this time, owing to the complications in the adult. The muscles and nerves of the eye pass directly through the organs and the nerve which supplies the electric tissue is in some way curiously involved with the oculomotor nerve, but no connection between the electric tissue and the eye-muscle tissue is evident, although the rectus inferior muscle has become so narrow as to appear as a mere thread and the rectus superior muscle is split into several parts by the passage of the electric nerve through it. The organ is bounded by the upper part of the muscle adductor mandibulæ, the muscle levator arcus palatini, and the anterior and posterior divisions of the muscle adductor arcus palatini.

The authors supposed the organs to have been derived from one of the bounding muscles, probably the muscle levator arcus palatini, since this has a more intimate connection with the organ than either of the other two, but the possibility was suggested of the tissue having arisen from the eye-muscles which pass through the organ. It was impossible to follow up either of these theories, however, without embryonic material, and a search for embryos was at once begun.

Opsanus and *Porichthys* lay their eggs in nests or easily available places. This did not prove true of *Astroscopus*, however, and Professor Dahlgren studied the habits of the fish to find if possible where to look for the young forms. He found the habits of *Astroscopus* to be essentially similar to those of an allied form, *Uranoscopus scaber*, a native of the Bay of Naples. This fish, as explained by Dr. Cerruti (**30**), lays a pelagic egg which at once floats from the bottom, where it is deposited, to the surface, where it rapidly forms an embryo that lives on the surface for many weeks and months. *Astroscopus* has a large pelagic egg which is laid in May or June and develops into a larva very rapidly. It lives on the surface 50 to 60 miles from shore and gradually works inshore as it approaches adult life. Toward the latter part of the summer the young fish, over an inch in length, moves from the surface to the bottom and seeks the sand, in which it burrows and spends the greater part of its life. With this information extensive towing work was undertaken, and through the kindness of Dr. Henry B. Bigelow, of the Museum of Comparative Zoology at Harvard University, and of W. W. Welsh and Lewis Radcliffe, of the United States Bureau of Fisheries, the results of the



Astroscopus guttatus, 20 mm. embryo.
Drawn from specimen by Bruce Horsfall.

towings by the steamer *Fish Hawk* and the schooner *Grampus* were examined, and a few embryos of different sizes were found, from which the following results have been obtained. Recognition and thanks are also due to Dr. A. G. Mayer and his associates in the Carnegie Institution of Washington who have assisted in procuring these specimens.

In a few cases it was difficult to determine whether the embryos were *Astroscopus* larvæ or those of a nearly allied form, *Kathetostomata*, which lives in deep water off the Atlantic coast; but since the adult *Kathetostomata* has been studied and found to possess no electric organ, there remains no doubt that these embryos which possess the first rudiments of the electric organ are examples of *Astroscopus*. The embryos were all photographed before sectioning to aid in the identification of the species, but since *Astroscopus guttatus* and *Astroscopus y-græcum* have very similar electric organs in the adult, it matters very little for this work whether the embryos are all of one species or not. Most of them have been identified as *Astroscopus guttatus*.

OBSERVATIONS.

The sectioned heads of these embryos have been handed over to me by Professor Dahlgren for the purpose of tracing the various steps in the development of this curious electric organ and to clear up such points concerning its innervation and blood supply as the material would allow. Some attention will also be given to the change in histological structures of the electric tissue during its development from the muscle tissues.

It quickly became evident that the electric tissue in the young embryos was being formed by the metamorphosing of certain parts of some of the eye-muscle, a phenomenon not hitherto met with and therefore of special interest.

STUDY OF AN EMBRYO 9.5 MILLIMETERS IN LENGTH.

The head of this embryo was sectioned serially and transversely and reconstructed with 2 mm. wax at a magnification of 500 (plate I). Some of the eye-muscles of this little embryo are already beginning to show a change, although no definite electric tissue has yet formed. The rectus externus, rectus internus, and rectus superior muscles show a definite darkened area on the side away from the eye-ball, which, upon examination, proves to be composed of cells smaller than the normal muscle-cells. Both the nuclei and the cytoplasm of these cells take the stain much more densely than the other cells, but this is the only sign of any modification of the cell structure (plate V, fig. 2).

Sections of a 4 mm. embryo were also studied with regard to this particular point, although the specimen was too poorly preserved for any more careful investigation. In this embryo areas corresponding to those mentioned above were found on the same muscles and with the same topographical relation to the muscle-cells. They had the same dense stain, but were very much smaller in area, as indicated in figure 1 of plate v.

A few embryos of the species *Batrachus tau* were then sectioned in order to observe the muscle-tissues, as it was thought possible that the smaller and more numerous cells might represent the generating area of the muscle itself and have no connection with the electric tissue. In these embryos such areas were found on several of the muscles, but a very interesting point lay in the fact that in every case this area of generating tissue lay on the opposite side of the muscle from that on which the electric organs of *Astroscopus* are derived, *i. e.*, on the side toward the eye-ball. In *Astroscopus*, however, this area corresponds in every case with the position from which the electric organ is to be generated; moreover, no such areas could be found in *Astroscopus* on the muscles not forming electric tissue. For this reason I feel certain that the darkened areas represent the first beginnings of electric tissue. The possibility also arises that in the darkened areas on the muscles of the young *Batrachus* we may have a step in the evolution of the electric organs in the toad-fish group.

The innervation of the eye-muscle in the 9.5 mm. embryo is perfectly normal and no nerve-endings are seen even to approach the electric tissue. A résumé of the normal eye-muscles, their origin, insertion and innervation will here be given for reference (Herrick 61 and Wiedersheim 94). The eye-muscles are six in number—rectus internus, externus, inferior, and superior, and obliquus inferior and superior.

The rectus internus, externus, and superior originate in the subcranial canal at the extreme caudal end of the basioccipital. The rectus externus originates in the extreme caudal end of the canal and runs dorso-laterad to its insertion on the base of the eye-ball. It receives the entire abducent or sixth nerve.

The rectus internus originates near the caudal end of the canal in a groove on the dorsal side of the parasphenoid bone and runs along the inner sides of the orbits to its insertion on the cephalic border of the eye-ball. It receives a branch of the oculomotor nerve, or the third nerve.

The rectus superior originates in the cephalic end of the subcranial canal from the parasphenoid, under the rectus internus, and from the membranous roof of the canal over the same. It runs close to the rectus internus to the end of the canal, turns dorsal and laterad, and crosses the rectus inferior and optic nerve to reach its insertion. It receives a branch of the oculomotor nerve.

The rectus inferior arises from the basisphenoid bone and runs over the rectus internus, rectus superior, and optic nerve to its insertion. It receives a branch of the oculomotor.

The obliquus superior and inferior muscles arise far cephalad from the internasal cartilage and run caudal to their insertions.

The obliquus superior arises farther cephalad than the interior muscle and is inserted on the dorsal surface of the eye-ball. The obliquus inferior originates a little farther back from the dorsal wall of the cartilage and lies nearer the median line. It passes mediad from the superior muscle direct to the ventral side of the eye. The obliquus superior is innervated by the entire trochlear or fourth nerve and the obliquus inferior receives a branch of the oculomotor.

The sixth or abducent nerve arises from a center in the medulla some distance from the median line near the lateral surface of the brain. It passes through a foramen in the cranial wall into the dorso-lateral angle of the subcranial canal and passes direct to the lateral face of the rectus externus, which it enters.

The trochlear or fourth nerve arises from a separate center in the hind-brain just caudal to that of the third nerve. It leaves the brain behind the optic lobes and continues intracranially a short distance, when it pierces the cranial wall, turns dorsad, and moves direct to the dorsal side of the obliquus superior.

The oculomotor or third nerve is a large nerve arising in the midbrain from the nucleus near the median line in part mesial of the large fasciculus longitudinalis dorsalis. There is no trace of the differentiation of the large electric motor-cells at this stage. The nerve leaves the brain and gives off its first branch to the obliquus inferior. Both branches pass at once through the cranial wall just mesial of the rectus externus. The branch to the obliquus inferior turns ventrad and passes the rectus externus, internus, and inferior on its way to the ventral face of the obliquus inferior. The main branch gives off another branch which divides and goes to the rectus internus and inferior, but the main branch itself divides again, both branches entering the rectus superior muscle, a point of significance in light of further investigations.

STUDY OF AN EMBRYO 14 MILLIMETERS LONG.

The 14 mm. embryo was sectioned serially and transversely, but was too poorly preserved for very careful study. The region of the electric tissue on the three muscles, rectus externus, internus, and superior, is now much more clearly defined and has considerably increased, but no indication of electric tissue or any other muscle can be found. The electric cells have enlarged slightly and can be identified by their multinucleate state as well as by their dense-staining capacity. The electric area is now as large as the muscle area in width, though not in length (plate v, fig. 3). Nerve-fibers do not yet approach the electric tissue on any of the eye-muscles.

STUDY OF AN EMBRYO 20 MILLIMETERS IN LENGTH.

The head of the 20 mm. embryo was sectioned serially and transversely and reconstructed in 1 mm. wax at a magnification of 500. This embryo was so well preserved that a very careful study has been made of it (plates II and III).

The electric area has become definite electric tissue and is now seen for the first time on the obliquus superior muscle, making it evident that the electric tissue is generated from muscles which are innervated by the fourth and sixth nerves as well as from some which are innervated by the third nerve. The three rectus muscles which contribute to the electric tissue have a common origin in the subcranial canal, the rectus inferior muscle having a separate origin. The obliquus superior arises at some distance from the three rectus muscles, but is the more dorsal of the two obliquus muscles. The fourth and sixth nerves take no part in the innervation of the adult electric organs. The organs are exposed dorsally, on the other hand, and only those muscles which are nearest the dorsal surface of the head have contributed to their formation, the two inferior muscles on the ventral surface not being concerned. *Torpedo* is the only other fish known to have the electric organs on the exposed or the dorsal surface, even though it occupies all the space between the dorsal and ventral surfaces. This tendency to develop the electric organs on the exposed or dorsal surface may be compared with the opposite tendency of fishes and other animals to develop their luminous organs on the ventral or protected surface, as shown in *Porichthys*. This correlation of the protective organ with the exposed surface and the attractive organ with the protected surface may be the result of natural selection, even though Darwin (37) considered electric organs to be one of the greatest difficulties in the way of his theory.

The electric cells have assumed the form of electroplaxes (plate V, fig. 4). They have enlarged to about six times the diameter of the ordinary muscle-cells; and the large and numerous nuclei have rounded up close to the membrane, although a few remain scattered in the cytoplasm. The cytoplasm itself has become peculiarly vacuolated and very definite striations have appeared. In longitudinal section each electroplax shows the ventral end indented into long, finger-like processes (plate VI, fig. 1). The striations across these processes are continuous, as though the cell had been cut through at this point after the striations had been formed instead of the processes having grown out. The striations are perfectly definite and pass straight across the cell with no indication of the myofibrillation. Long, narrow vacuoles appear at intervals in the cytoplasm of the indented or evaginated end, while the dorsal end of the cytoplasm is filled with vacuoles of a smaller, rounder nature. This indentation is the beginning of the formation of the ventral papillæ of the adult electroplax.

Vacuoles are formed in cells when changes in surface tension occur such that the pressure inside the cell-wall is greater than that of the surrounding medium. The electroblasts are growing at an abnormal rate in the longitudinal direction and not at all in the other direction, so that the pressure inside the cell must be tremendous. If this tension reaches a certain point, the vacuoles may increase in size until they burst through the cell-wall. That this is happening in these electroblasts is shown by the long, narrow shape of the vacuoles at the ventral end, where many have already broken through to form the papillæ. This explains why the striations at this end appear to be broken across instead of being separate striations formed for each papilla. It also explains why in the adult electroplax many of the papillæ have connecting bridges of the same material as themselves (Dahlgren **36**), for in such cases the vacuoles have merely persisted instead of breaking through. The tension at the ventral end far exceeds that of the dorsal end, for the small, round vacuoles finally disappear without breaking through the wall and the dorsal surface becomes straight and smooth, the dorsal cytoplasm homogeneous and unvacuolated.

The electric organ develops in four parts on each side of the eye, each of which is called an electric organ for convenience during its development. The rectus externus is the largest of the six eye-muscles and has a proportionately large electric organ. It is the first of the four to begin development. The central part of the tissue forms a broad band lying close to the muscle and curving from its dorsal to its ventral surface in a spiral. The ends of the tissue separate from the muscle ventrally, just caudad to the origin of the muscle, running caudad and ventrad, and dorsally, near the point of insertion, running craniad. This separation of the organs from the muscle with the turning and bending of the organs is accomplished by the elongation of the electroblasts at the point of separation. The growth of the cells, therefore, begins at either end of the organ and later progresses toward the center. Also, the electric organ of the rectus externus muscle, which was the first to begin development, is the first to begin to separate from the muscle-tissue (plates II and III, *R.* ex.).

The long, narrow electric organ of the rectus internus lies close to its surface and curves spirally from a point cranial and medial around the dorsal to the ventral side of the muscle. Dorsally the organ remains closely attached to the muscle, but ventrally it separates to run caudad a short distance. It is the second of the four electric organs to begin development and the second to begin to separate from the muscle (plates II and III, *R. int.*).

The electric organ of the rectus superior muscle lies close to the median side and does not curve around it nor separate from it. The obliquus superior is the last of the four muscles to develop an electric organ, which is correspondingly small, forming a short, broad band, caudad and close to the muscle, about halfway between its origin and insertion (plates II and III, *R. su.*, *O. su.*). In the figures the electric area is stippled.

The number of electroblasts do not increase after once being formed. Each electroblast is the product of a single cell and each electroplax is the product of a single electroblast, so that the amount of electric tissue to be contributed by the various muscles has already been determined. It was most unfortunate that no embryo could be obtained representing a stage between that of the 20 mm. embryo and of the 33 mm. embryo, as it is between these two stages that the actual separation of the organs from the muscles takes place. The position of the electric organs in the 20 mm. stage, however, goes a long way toward explaining the real process, so that we are safe in drawing pretty definite conclusions without the intermediate stage.

Except that the nerves branch much more profusely in all of the muscles, no change has been noted in any but that branch of the oculomotor nerve which goes to the rectus superior. As noted above, the main branch of the oculomotor goes to the rectus superior in two divisions. The larger of these two divisions enters the muscle somewhat posterior to the smaller one, and is seen to have enlarged to nearly twice its original diameter. The small branch goes to all parts of the muscle, but the large one goes to the middle of the muscle, where it divides into many parts, all but one of which are seen to approach the electric organ of this muscle and stop. There is a considerable space between the muscle and the electric tissue, and the axones of the nerves must cross this space to enter the electric organ. Although the material was not favorably fixed for the study of nerves, a few fibers could be traced across the intervening space and into the electric organ. Unfortunately, the nerve-endings could not be seen in any of my material and I have had to pass over this point until more material can be obtained. The last division of this branch, which does not approach the electric organ, passes directly through the rectus superior muscle and can be traced ventrally and mesad until it approaches the electric organ of the rectus internus muscle. It was impossible to trace any of the fibers of this branch into the electric organ, but the nerve itself could be traced until it touched the electric tissue, so that there can be no doubt that the axones do pass in and that with better material they could be seen to be distributed among the electroplaxes of this organ as well as that of the rectus superior. This is the first indication that the nerve which innervates the rectus superior muscle is to innervate the electric organs also, but it is not yet clear that it is the only nerve which will do so, although it is clear that the electric organ of the rectus superior is not the only one to be innervated by it. Later it sends branches to all four of the electric organs.

A few electric motor-cells are found in the motor nucleus of the third nerve. It is of interest in this connection that the rectus superior muscle is neither the first of the muscles to develop electric tissue nor does it contribute the largest amount of tissue to the organs. On the other hand, it does not contribute the smallest amount either, so there is no apparent reason why the nerve of this particular muscle should be called on to do all the work. It might have been expected that the sixth nerve, which innervates the rectus externus muscle, would contribute to the electric organs, since that muscle has the largest of the four organs, or (on the other hand) that the trochlear or fourth nerve to the obliquus superior muscle would contribute, since that muscle has the smallest amount of electric tissue. As a matter of fact, subsequent study has shown that there are no electric motor-cells to be found anywhere in the brain except in the nucleus of the third nerve, no fibers from any other nerve or from any other branch of that nerve even approaching the electric tissue. When the third nerve emerges from the cranial wall the first of the muscles which it is to innervate which crosses its path is the rectus superior; it is also the first muscle which it enters. Whether this fact has any significance for the fact that this particular branch forms the electric nerve or not, I can not say, but no other explanation presents itself.

The circulation in the head of the 20 mm. embryo appears to be perfectly normal, presenting no points of difference from that of other non-electric toad-fishes. The external carotid artery runs craniad just dorsal to the rectus inferior muscle, and sends off small branches to the various eye-muscles on the way to the eye-ball; it then runs between the two oblique muscles and craniad below the eye-ball. The vessels to the eye-muscles send no branches to the electric organs, either before or after entering the muscles. The ophthalmic artery branches from the pseudobranch to run craniodorsad to the eye-ball, which it enters with one of the ciliary nerves from the fifth cranial. It gives off a small branch just dorsal to the obliquus inferior muscle, which anastomoses with the corresponding artery on the other side, but this branch has no connection with the electric organs.

STUDY OF AN EMBRYO 33 MILLIMETERS IN LENGTH.

The head of the 33 mm. embryo was sectioned serially and transversely and reconstructed in 1 mm. wax at a magnification of 45. Four well-developed electric organs appear, completely separated from the eye-muscles (plate IV). They are quite separate from each other dorsally and ventrally, but in the center they are so closely folded together that at times only a thread of connective tissue separates them and without the previous studies it would be impossible to determine from which muscle the different organs have been generated, or, in fact, that they had been generated from muscle-tissue at all. It can be seen, however, that the four organs are still distinct. Together they occupy the entire space between the eyes and the brain, and although they have been derived from only the dorsal muscles they extend almost as far in the ventral direction as in the dorsal, the only difference being that the ventral ends of the organs are narrower and more pointed than the dorsal ends (plate VII, fig. 2).

The electroplaxes have assumed an elongate shape, being in general from three to four times as long as they are broad. They still have quite a thick appearance, however, for while they have been growing rapidly in one direction they have not become any narrower in the other, the width of these electroplaxes being only slightly less than that of the original electroblast. They are arranged in parallel rows, but since all four of the organs slant in a different direction, the rows are not parallel to the surface of the embryo, and on the whole they are only fairly regularly arranged. Ventrally the surface is entirely cut up into papillæ and many vacuoles are seen in the process of forming more (plate VI, fig. 2). The dorsal area shows a decided change in structure. The surface has become flat and smooth, the indefinite area of the 20 mm. embryo having completely disappeared. A few scattered vacuoles persist, but they are very small and round. The three layers of the adult electroplax have now been formed: the ventral layer, which is nutritive in function, with scattered nutritive nuclei, many papillæ, vacuoles, and perfectly straight striations; the middle or intermediate layer, with no nuclei and no vacuoles, but with an abundance of striations; and the dorsal layer, with a few small, round vacuoles, a few pretty definite striations on its ventral edge, and a very regular series of oval electric nuclei. The rods described by Dahlgren and Hughes have not yet appeared.

In the previously described embryo some of the nuclei had rounded up close to the membrane, while others remained free in the cytoplasm, but no difference in the structure of the two kinds of nuclei could be noted (plate VI, fig. 1). It is now evident that those nuclei which were close to the edge of the membrane have become flattened to fit the space, so that they appear quite oval in shape, while the nuclei which remained in the cytoplasm have become the round nutritive nuclei. They are less numerous than the electric nuclei and are not regularly placed in a series, but scattered through the cytoplasm of the nutritive layer. The electric nuclei have each a single nucleolus from which the chromatin runs out in fine strands through the caryoplasm, whereas the nutritive nuclei have several aggregates of chromatin but no well-defined nucleolus.

The electric nerve is now well developed from the branch of the oculomotor which supplies the rectus superior muscle. It has assumed such large proportions that the rest of the third nerve has a diameter only one-fourth that of the electric nerve, and the third nerve itself is three or four times as wide as either of the other nerves which supply the eye-muscles. The third and electric nerve leave the brain together from the same center, which is now filled with the large electric nuclei, occupying much more space than the ordinary third nucleus (plate VII, fig. 5). They appear mesial, dorsal, and ventral of the fasciculus longitudinalis dorsalis. The cells are large and egg-shaped, with very granular cytoplasm. A large nucleus lies at the broad pole of the cell-body. It is fairly round in shape and has a few strands of chromatin running through it, as well as a very small aggregate of chromatin which is fairly persistent. At the pole of this nucleus corresponding with the broad pole of the cell is a very definite round nucleolus with a vacuole on one side. This body takes the chromatin stain heavily, while the rest of the nucleus takes it very lightly and the cytoplasm of the cell with only medium density. In the adult the cells measure 32 by 56 microns at their broadest point.

The third nerve branches from the electric nerve immediately upon leaving the brain, but continues to run close to its side until it has passed through the cranial wall (plate VII, fig. 6). In speaking of the electric nerve, it will be understood that that branch of the third nerve which goes to the rectus superior is included and, for that matter, that the electric and third nerve are one from their origin, but are given separate names for convenience. The other branch will be known as the third nerve. After passing through the cranial wall they go direct to the rectus superior muscle. The third nerve then passes laterad around the muscle, dividing at the same time into three branches for the three muscles which it is to innervate (plate VII, fig. 4). The small ciliary nerve to the fifth cranial has up to this time been in such close connection with the third nerve as not to be distinguishable from it, but now separates and can be traced into the sclera of the eye. The three branches of the third nerve pass directly through the electric organs in their passage to the muscles, but otherwise their behavior is perfectly normal and as described for the 20 mm. embryo.

The electric nerve passes with its entire bulk into the rectus superior muscle, the branch which is to innervate the muscle itself being separated from it just previous to entering. The nerve is twice as wide as the muscle itself, so that it tears the tissue of the muscle apart as it enters, and when it divides in the muscle its branches separate the fibers until they lose all connection with each other. One very small branch of the electric nerve innervates the muscle; the rest pass out and go in all directions through the electric organs, dividing and redividing as they go until the branches are no wider than ordinary nerves. Some of the branches turn back dorsally, go around the organs, and enter from the dorsal end; others pass directly through the organs mesially and laterally and enter the organs from the ventral end, while still others push their way between the organs in a ventral direction, entering the organs on either side as they go, so that no corner of the four electric organs escapes their reach, no matter how remote. It is possible to trace each nerve to the particular electroplax which it innervates, but since all are branches of the same nerve this would have no significance. The nerves enter the electric organs between the electroplaxes which they enter on the dorsal surface. They can be seen to reach all points on the surface, but the nerve-endings could not be observed because of the unsuitable fixation.

The fourth nerve can be seen to arise from its separate center in the brain and to have no connection with the electric nerves.

The circulation has made rapid advance since the 20 mm. stage. Although the blood-vessels in most instances differentiate more rapidly than nerves, the arteries to the electric organ are not formed until after the innervation has been completed. In the 33 mm. embryo the branches of the carotid which nourish the eye-muscles have begun to give off small vessels at intervals which enter from the ventral surface. A large branch is given off from the vessel which goes to the rectus superior and another from the vessel which goes to the rectus inferior; other smaller branches are contributed by the vessels which go to the other muscles. A point in this connection is the fact that those muscles which have no part either in the formation of the electric organ or in their innervation do contribute toward their nourishment, so that all of the six eye-muscles are in some way involved.

The rectus inferior muscle has become so reduced that its widest portion is no wider than the nerve which innervates it (plate I, *R. inf.*). It passes directly through the electric organs, but at its point of origin on the basiosphenoid bone there are no muscle-fibers at all, a thin connective-tissue tendon attaching it to the bone. At a point slightly dorsal and some distance posterior to its origin muscle-fibers begin to appear and the muscle grows gradually wider. It is small wonder that the eyes of *Astroscopus* do not have the power of movement of an ordinary fish when the rectus inferior muscle has degenerated to a mere tendon and the rectus superior has been split into several parts by the huge electric nerve.

STUDY OF AN EMBRYO 45 MILLIMETERS LONG.

The head of the 45 mm. embryo was sectioned serially and transversely, but the changes showed so plainly that no reconstruction was made of it. It is now impossible to trace the divisions between the separate electric organs, there appearing one very large organ which occupies the entire space between the eye and the brain and extends from the dorsal surface to a point not far from the ventral surface of the embryo, completely inclosing the eye-muscle (plate VII, fig. 3 and figs. 5–10).

There is no change in either the innervation or the circulation of this embryo.

The electroplaxes have now become very narrow and elongate and are arranged in parallel rows, strictly parallel except at the extreme edges or where a muscle passes through. Ventrally the surface is completely broken up into papillæ by the vacuoles, a few of which still persist, forming bridges across from one to the other. The electric layer has completely lost its vacuoles, but no black rods can be seen. The striations of the nutritive layer have taken on an entirely new appearance, differing but little from those in the adult. They no longer pass straight across the cell, but are broken up into a large number of small, curving striations, running in all directions and over and under each other in a manner which gives the effect of a maze. They are divided into groups, however, each group being composed of a few very definite, sharp lines, strictly parallel to each other, but with no relation to the lines of any other group. The lines of one group are all in one curved plane. They may lie over or under another group, but not between the lines of another group. The optical effect of these lines is like that of normal muscle striations, there being a dark area close to a light area (plate VI, fig. 3, *adult*).

It can be seen in section that the curious infoldings and overlappings of electroplaxes are beginning to form. Some of the electroplaxes run along parallel to the rest for some distance and then drop down perpendicularly one, two, or three rows, when they turn and continue in a straight line between two other electroplaxes. The electroplaxes between which they pass come up close to them so as to fill in the spaces, but whether they have been cut in two by the dropping of the electroplax or whether it has merely passed between two shorter electroplaxes could not be determined. This peculiar phenomenon has not been described for the electric organs of any other fish, but a possible explanation for this lies in the fact that the electroplaxes of *Astroscopus* must turn as they grow in order to properly orient themselves with reference to each other and to the body-surface, so that it is not surprising to find that some of them have become twisted in this particular manner and remain so. In all the other electric fishes which have been studied up to the present time, with the exception of *Malopterurus*, the electric tissues have been derived from muscles which are so oriented from the start that the electroplaxes come off from them in the exact position best suited for their purpose. They have, therefore, only to increase in length and to multiply their nuclei in order to form the regular parallel rows which are invariably found in the adult organ.

It is not mere chance, however, that the electric organs are formed of parallel electroplaxes any more than it is by chance that the electroplaxes are long, narrow cells with a definite polarization. In order to get an electric current the cells must be parallel, they must be polarized, and the current is more effective if they be long and narrow, since then the resistance to the current is less. In *Astroscopus* the electroplaxes are formed from four different muscles, running in four different directions. The necessity for orientation is therefore evident. The process takes place between the 20 mm. and the 45 mm. stages. In the 20 mm. embryo the electric tissue is still a part of the muscles, though separating from them at the ends. In the 33 mm. embryo the organs are quite separate from the eye-muscles, but still exist as four separate organs, each organ lying in the position in which it came off from the muscle, so that they lie in four different directions. The electroplaxes of each separate organ lie approximately equal to each other in the middle, but on the edges they may be found in all stages of orientation, some of them having assumed the shape of horse-shoes, crescents, etc.

From the first the electroplaxes are very evenly spaced in the longitudinal direction and closely interlocked in the other. The cells elongate as they turn and the two processes occurring at the same time probably account for the curious overlapping of the electroplaxes described above. There appears to be a much larger number of the short electroplaxes in the 33 mm. stage than there are of the longer electroplaxes of the 45 mm. stage—doubtless another expression of this peculiar turning which enables the microtome knife to cut them in various planes, so that there appears to be a larger number of them than there really is. This fact also lends grounds for the idea that the electroplaxes in dropping from one row to another may cut through an otherwise single electroplax, making two where there was but one.

The process of orientation has been practically completed by the 45 mm. stage when the electroplaxes are found in their final position, parallel to each other and to the body-surface. At the extreme edges the electroplaxes never do completely straighten out, for it would seem that the body is not wide enough to accommodate the rapid growth of the electroplaxes, which continue to grow as long as the growth of the fish continues. Another place where the electroplaxes do not become strictly parallel is where the muscles pass through the electric organ. There is always a considerable amount of space around the muscle and the electroplaxes bend in toward each other at the edges, making a wall around them. At the extreme dorsal and ventral surfaces of the organ, also, the electroplaxes are crowded up closer than usual. With these few exceptions, by the time the fish is 45 mm. long the arrangement of the electroplaxes is strictly parallel and at even distances from one another.

The extraordinary regularity of the spacing is a very striking point. The width of the area between the electroplaxes does not vary more than 0.5 micron anywhere in the organ. Looking to the other electric fishes for comparison, we find that, with the exception of *Malopterurus*, all exhibit this same remarkable regularity, but that in some forms the electroplaxes are much wider apart than in others. For instance, in *Astroscopus* there is a distance of 12 microns between every two electroplaxes, while in the Rajidæ the distances vary in the different species from 15 to 75 microns. In *Torpedo occidentalis* there are 5 microns between the electroplaxes, in *Torpedo ocellata* 4 to 8, and in *Gymnotus* 15; while in the Mormyridæ the space is very large.

EXPERIMENTAL.

It was hoped at one time that the shock of *Astroscopus* might be accurately measured and so given its place among the other electric fishes. One living specimen was sent from Norfolk, Virginia, to the Princeton aquarium, where, with the aid of a physicist, Professor Adams, of Princeton University, a few experiments were carried on. Unfortunately the specimen was so weak from exhaustion when it arrived that only fatigue phenomena could be observed, and since the specimen never revived it was impossible to measure the current with any accuracy. It was possible, however, to make a few observations of value. The direction of the current was tested and found to agree with Pacini's law (76)—in other words, to pass from the motor to the nutritive layer of the electroplax. With the exception of *Malopterurus*, all electric fishes examined in this regard have been found to agree with Pacini's law. The exception of *Malopterurus* in this respect is probably not a serious one, since it was already known to differ from other electric fishes in nearly all respects except the power to give electric shocks and in its possession of the characteristic transparent jelly-like areas.

In *Gymnotus* and *Mormyrus* (Garten 49), where the electric layer is posterior to the nutritive, the current passes from tail to head. In *Raja*, which has its electric area on the anterior side, the current passes from head to tail. In *Torpedo* the electric disk is on the ventral side and the current passes from ventral to dorsal, and now it is seen that in *Astroscopus* the current passes from dorsal to ventral, the electric surface being on the dorsal side. No living specimen of *Gymnarchus* has been obtained, but the general resemblance of the organs of this fish (both in structure and origin) to those of the other Mormyridæ leaves very little room for doubt that the current passes from tail to head, as in those fishes.

The current entering on the dorsal side in *Astroscopus* makes the dorsal surface negative to the ventral; the nerve-endings are therefore all on the negative side. Although the organs of the various fish are innervated in quite different ways, yet in every case the entire electric surface is covered with nerve-endings, so that if it be the nerve and not the organ which generates the electricity the amount of electric surface and the arrangement of the surfaces would still be significant.

DISCUSSION.

Bernstein and Tschermak (13) have suggested that the electrical discharges may be produced by different concentrations of sodium chloride in the electroplaxes and in the intervening connective tissue, a theory which necessitates the presence of a membrane between the two solutions which is permeable to one kind of ions and not to the other. Such a membrane is not known to be present, but neither is there any evidence against its existence, and without it the bio-electric current would appear to be arising without the ionization of anything. The principle of the bio-electric current is that normal sodium chloride and concentrated sodium-chloride solutions coming in contact diffuse their ions at different rates, causing currents in small amounts, just as they do in a muscle where such a membrane is known to exist.

Engelmann (39) has compared the thin, dark areas (electric) of the electric organs with the isotropic portion of the muscle-tissue, and the thick, light area (nutritive) with the anisotropic portion. The action current of an electric organ is the same as that of an ordinary striated muscle, except that the arrangement of the electroplaxes in a column makes appreciable an otherwise insignificant current. The action current of muscle can only be detected by the galvanometer, while that of an electric organ can be felt by the hand. Muscle, however, can be stimulated to give off electricity by mechanical, thermal, and chemical means as well as by the stimulation of the nerve. It can also be effectively paralyzed by small doses of curare and atropin, so that the nerves themselves are not affected, whereas electric organs can not be paralyzed unless the nerves are rendered powerless.

The strength of the shock in fishes is made possible by the arrangement of the electroplaxes in a column. In a battery, when the cells are arranged so that all the positive poles are connected together and all the negative poles together, they are said to be arranged in parallel, and when each positive pole is connected with a negative one, they are said to be arranged in series. According to Ohm's law, $C=\frac{E}{R}$, where $C=$ the strength of the current measured in amperes, $E=$ the electromotive force measured in volts, and $R=$ the resistance measured in ohms. Now, when the cells are arranged in series the electromotive force of the combination is the sum of the electromotive forces of the several cells, and the resistance of the cells is the sum of the resistances of the separate cells, providing high voltage and therefore high shocking power, but little current. When the cells are arranged in parallel the electromotive force is the same as that of one cell, while the resistance is less, being inversely proportional to the number of cells, thus providing a large current but low shocking power. By combining these two arrangements—that is, by putting the cells of one group in series and then joining several such groups in parallel—various degrees of shocking power with much or little current may be obtained (Kimball 67).

In the case of the electric fishes it would seem that the electric organ itself forms the battery in which the different concentrations of sodium chloride form the poles, and in which the electroplax takes the place of the battery cell; or if the organ be composed of columns, then each column is a complete battery in itself, in turn acting as a cell in the larger battery, the electric organ. The surrounding medium, salt or fresh as the case may be, forms the conductor, and the nerves form the key which closes the circuit. When the nerve is stimulated the circuit is closed and the organ gives a shock. The organ being self-excitatory, is peculiarly adapted to give interrupted shocks after the manner of an induction coil, for when the nerve has been restimulated by the first shock it will go on opening and closing the key until the organ is worn out by fatigue. Gotch has shown that it is as impossible to fatigue the electric tissue as it is to paralyze it unless the nerve be fatigued (50).

In nearly all the electric fishes the electric organs are divided into columns. In *Torpedo ocellata*, for example, there are 450 columns in each electric organ and 400 disks in each column (Gotch 50). The current in this fish goes from ventral to dorsal, the ventral surface being negative to the dorsal one. In each column the disk nearest the ventral surface of the body will present its negative side to the surface, while the disk nearest the dorsal end will present its positive side, so that to complete the circuit between these two points it will be necessary to connect a positive with a negative surface. Thus the electroplaxes of each column will be arranged in series, so that the voltage of a column will be equal to that of all 400 disks combined, the amperage that of one. The 450 columns of the electric organ, however, are arranged in parallel, since all the surfaces facing the dorsal side of the body will be positive and all those facing the ventral side will be negative. In *Torpedo ocellata*, therefore, the amperage will be 450 times that of one column, the voltage 400 times that of one electroplax, a condition providing for a large amount of electricity and at the same time for a comparatively strong shock. Salt water is a million times better conductor than fresh water (DuBois-Reymond), thus furnishing very little resistance, so that *Torpedo*, although a fish of sluggish habits, has everything in its favor for effectively shocking its enemies, high voltage, high amperage, and low resistance.

In *Gymnotus* there are four electric organs lying lengthwise in the body and divided into a variable number of columns, each of which may have as many as 10,000 electroplaxes. Here, as in *Torpedo*, the columns as well as the four organs themselves are arranged in parallel and the electroplaxes in series. The resistance of fresh water is very great, but the arrangement of the columns in parallel provides for a larger amount of electricity and the extraordinary number of electroplaxes in series overcomes the resistance sufficiently to give a very strong shock. In *Malopterurus* also the arrangement of the disks is an extensive one, even though not apparently arranged in either parallel or series, and so is adapted to the high resistance of the water.

It has been calculated, (Schönlein [83]) that the electromotive force in one disk of *Raja* is 0.03, in *Torpedo* 0.04, and in *Malopterurus* may reach as high as 0.45 volt. These results show that aside from the number of electroplaxes there is a specific difference in the power of the individual electroplax. It was most unfortunate that the voltage of *Astroscopus* could not be determined, as it might then be possible to explain why *Astroscopus*, which has as many as 200 electric layers in each organ, arranged in series, and with no apparent resistance, should still have a shock so much weaker than *Torpedo*, as all reports would indicate.

The organ of *Astroscopus*, unlike the other electric organs, is not divided into columns, the diameter of a single electric layer being equal to one-third the diameter of the fish. When a current passes through a copper wire the resistance to the passage of the current is inversely proportional to the diameter of the wire. Therefore, were the diameter of a single electric layer in *Astroscopus* equal to the sums of the diameters of the 450 columns in *Torpedo*, the amperage of *Astroscopus* would be equal to that of *Torpedo* with only one column. As a matter of fact, the diameter of the organ in *Astroscopus* is much less than that of the columns in *Torpedo*, although the exact amount has not been computed. The amperage is therefore low, but the voltage being the sum of the 200 electric layers should be a little less than half that of *Torpedo ocellata*. Since it is known to be less than that, it must be that the qualitative differences in the structure of the electroplaxes themselves may offer various grades of resistance and so account for otherwise inexplicable differences in voltage. Still, the organ of *Astroscopus* has been measured only in small and much fatigued fishes.

A comparison of the marine electric fishes demonstrates that with the increase in voltage there is correlated not only an increase in the number of columns and in the electroplaxes in a column, but also a decrease in the width of the electroplaxes and in the area between them. In the fresh-water fishes the same general principles hold, except that in *Gymnotus* the electroplaxes are only slightly thinner than in the Mormyridæ. The thickness in *Mormyrus* is 450 microns, in the Rajidæ from 300 to 700, and in *Gymnotus* 200, in contrast to 35 in *Astroscopus* and 15 in *Torpedo*. In comparison with the number of the electroplaxes in a column, however, the electroplax in *Gymnotus* is not a great deal thicker than that of *Torpedo*, so it is possible that the amount of material may affect the strength of the shock by offering some resistance to the current.

It has been noted that in the so-called weak electric fishes the nutritive layer of the electroplax is very definitely striated, as though it had not lost all relation to the original muscle-cell. Also, in the Mormyridæ the electric organs of some of the forms are connected to the muscle in the adult instead of being completely free as usual. In *Astroscopus* the striations are as definite as in the weakest fishes and so numerous as to be seen up into the electric layer. In *Torpedo* and *Gymnotus*, on the other hand, the electroplaxes have no structures to remind one of their muscular origin. In *Torpedo* they exist in the embryo only and are mere fibrillations, which in the case of *Gymnotus* are so reduced as to be recognized as striations only from a comparative study of the other forms. Are we here witnessing one phase in the evolution of electric organs, and are we justified in making these comparisons between such widely unrelated forms? We can not, of course, consider the transition from a mormyrid to one of the Rajidæ a case of natural selection, but if selection is taking place separately in each of the groups we may say that in the Mormyridæ the evolution of the electric organs has advanced to a much less degree than in *Torpedo* or in *Gymnotus*, and the lack of transitional stages in the groups themselves may not mean that they do not or have not existed. In the Rajidæ a very definite series exists, from forms with wholly undifferentiated electric tissue to those with definite electroplaxes, and not all torpedoes have shocks of the same intensity. It is probable, therefore, that the appearance of electric organs in the separate groups is a case of parallelism, evolution having taken place independently in each individual group. This parallelism may be accounted for by assuming that the evolutionary processes are continually working on the minute electrical discharge known to accompany the activities of muscle-cells, and that under proper environmental stimulus any fish might develop similar organs. What external factors initiate this development are not apparent, since electric organs appear to have developed simultaneously in fishes which lie buried in the sand on the bottom of the ocean and in fishes which swim freely about in both fresh and salt waters. The position of the organ in the body of the fish is individually adapted to the mode of life, those fishes which are commonly attacked from above developing electric organs in a dorso-ventral position, while those which swim freely in the water have antero-posterior electric organs. The primary use of the organ is probably that of protection, the capturing of food being a secondary adaptation.

SUMMARY.

1. Each of the two electric organs of *Astroscopus guttatus* is originally composed of four separate elements, each derived from one of the six eye-muscles. The rectus inferior and obliquus inferior muscles are the nearest to the ventral surfaces of the animal and are the only ones which do not contribute any electric tissue to the formation of the organ.

2. The four organs begin to differentiate before the embryo is 14 mm. in length. The first indication of the change is a slight darkening of the muscle-cells on one edge of the muscle, followed by a multiplication of nuclei and the rapid growth of the cells. No such areas form on muscles which do not form electric organs.

3. The electroblasts are completely differentiated by the 20 mm. stage and the organs have separated from the muscle by the 35 mm. stage.

4. Between the 33 mm. and the 45 mm. stages the four organs, now separated from the eye-muscle, orient themselves until their constituent electroplaxes become parallel to the dorsal and ventral surfaces of the body of the fish and unite to form one large organ with a diameter equal to one-third the diameter of the head of the fish.

5. Each electroplax is formed by the modification of a single myoblast cell.

6. The nuclei multiply and differentiate to form electric and motor nuclei. The electric nuclei are wide and flat, and arranged in a regular series on the negative surface of the electroplax. They have a definite nucleolus and faint chromatic threads. The nutritive nuclei are large and round, with several aggregates of chromatin but no definite nucleolus.

7. The cytoplasm differentiates into electric and nutritive layers.

8. There are no easily visible myofibrillations, but the longitudinal striations persist in the nutritive layer and are broken up into short, curved striations, very definite in outline. They can be traced into the electric layer.

9. The vacuoles of the dorsal surface disappear, but those of the ventral surface break through the membrane to form the papillæ and the protoplasmic bridges which characterize the nutritive surface.

10. The electroplax is 35 microns in width, one-third of which is electric and two-thirds nutritive material. It is twice as wide as the electroplax of *Torpedo*, but only half as wide as that of the weak electric fishes.

11. The electroplaxes are arranged in a parallel series, but some are carried down one or more layers in the process of orientation, so that they form a part of the lower layers.

12. The electric organ is innervated by that branch of the oculomotor nerve which supplies the rectus superior muscle. It begins to differentiate during the 20 mm. stage and is complete by the 33 mm. stage.

13. The electric and third nerves leave the brain together from the same nucleus, but separate before passing through the cranial wall. The electric nerve passes bodily into the rectus superior muscle, the fibers of which are torn apart by the process.

14. The branches of the nerve reach every corner of the electric organ and end on the dorsal surface of each electroplax.

15. The electric motor-cells are formed in the region of the third nucleus, mesial, dorsal, and ventral, of the fasciculus longitudinalis dorsalis. They are large, egg-shaped cells with prominent nucleus and nucleolus.

16. The blood supply of the electric organ is furnished by vessels from those branches of the external carotid artery which go to the six eye-muscles.

17. The blood supply does not begin to differentiate until the embryo is nearly 33 mm. long, and is not complete until the 45 mm. stage. It is the last of the structures connected with the electric organs to begin development.

18. The electric organ of *Astroscopus* can be stimulated by touch, and by chemical or electrical stimuli applied to the skin. It is also probably operated from some of the optic centers.

19. The current passes from the dorsal to the ventral surface in accordance with Pacini's law.

BIBLIOGRAPHY.

1. Abbot, 1880, Proc. Nat. Hist. Soc. Phila.
2. Babuchin, 1872, Ueber die Bedeutung und Entwickelung der pseudoelektrischen Organe. Ctbl. f. d. med. Wiss., X, 545–548.
3. ———, 1876, Uebersicht der neuen Untersuchungen ueber Entwicklung, Bau, und physiologische Verhältnisse der elektrischen und pseudoelektrischen Organe. Arch. f. Anat. u. Physiol., 501–542.
4. ———, 1877, Beobachtungen und Versuche am Zitterwelse und Mormyrus des Niles. Arch. f. Physiol., 250–274.
5. ———, 1882, Die Säulenzahl im elektrischen Organ von Torpedo marmorata. Ctbl. f. d. med. Wiss., XX, 866–869.
6. ———, 1882, Ueber die Präformation der elektrischen Elemente im Organ der Zitterfische. Arch. f. Physiol., 414–419.
7. ———, 1883, Zur Begründung des Satzes von der Präformation der elektrischen Elemente im Organ der Zitterfische. Arch. f. Physiol., 239–254.
8. Ballowitz, E., 1893, Ueber den Bau des elektrischen Organes von Torpedo mit besonderer Berücksichtigung der Nervenendigungen in demselben. Arch. f. mikr. Anat., XLII, 459–568.
9. ———, 1897, Zur Anatomie des Zitteraales mit besonderer Berücksichtigung seiner elektrischen Organe. Arch. f. mikr. Anat., L, 686–750.
10. ———, 1897, Ueber den feineren Bau des elektrischen Organs des gewöhnlichen Rochen. Anat. Hefte, 1. Abt., VII, 283–375.
11. ———, 1899, Das elektrische Organ des afrikanischen Zitterwelses.
12. Bean, 1903, Catalogue of the fishes of New York. N. Y. State Mus. Bull. 60.
13. Bernstein, J., und A. Tschermak, 1904, Ueber das thermische Verhalten des elektrischen Organes von Torpedo. Sitzber. d. k. preuss. Akad. d. Wiss. Phys.-math. Kl., 301–313.
14. ———, ———, 1906, Untersuchungen zur Thermodynamik der bioelektrischen Ströme. II Teil. Ueber die Natur der Kette des elektrischen Organos bei Torpedo. Arch. f. d. ges. Physiol., CXII, 439–521.
15. Biedermann, W., 1895, Elektrophysiologie.
16. Bilharz, Th., 1857, Das elektrische Organ des Zitterwelses.
17. duBois-Reymond, E., 1877, Gesammelte Abhandlung, Bd. II.
18. ———, 1883, Ueber sekundär-elektromotorische Erscheinungen an Muskeln, Nerven, und elektrischen Organen. Sitzber. d. K. preuss. Akad. d. Wiss., Phys.-math. Kl., 343–404.

19. duBois-Reymond, E., 1884, Lebende Zitterrochen in Berlin. Sitzber. d. k. preuss. Akad. d. Wiss. Phys.-math. Kl., 181–242.
20. ———, 1887, Lebende Zitterrochen in Berlin. Arch. f. Physiol., 51–110.
21. Boll, F., 1874, Ueber elektrische Fische. Samml. gemein. Wiss. Vorträge, IX Ser., Heft 210.
22. Breuer, J., 1905, Ueber den Galvanotropismus bei Fischen. Sitzber. d. k. Akad. d. Wiss. Wien, Math.-naturw. Kl., CXIV, 27–56.
23. Brünings, W., 1903, Beiträge zur Elektrophysiologie, I. Arch. f. d. ges. Physiol., XCVIII, 241–283.
24. ———, 1907, Beiträge zur Elektrophysiologie, III. Arch. f. d. ges. Physiol., CXVII, 409–460.
25. Burdon-Sanderson, J., 1898, Electrical response to stimulation of muscle, pt. II, Jour. Physiol., XXIII, 325–358.
26. ———, 1900, The mechanical, thermal, and electrical properties of striated muscle. Schäfer's Text-book of Physiology, pt. 2, 352–450.
27. ———, and Fr. Gotch, 1888, On the electrical organ of the skate. Jour. Physiol., IX, 137–166.
28. ———, ———, 1889, On the electrical organ of the skate. Jour. Physiol., X, 259–278.
29. Cavendish, 1776, Phil. Trans. Roy. Soc. London, LXVI, pt. 1, 196–225.
30. Cerruti, A., Mitt. Zool. Sta. Neapel.
31. Colladon, 1835, Geneva.
32. Dahlgren, U., 1914, Origin of the electric tissues of Gymnarchus niloticus. Carnegie Inst. Wash. Pub. No. 183.
33. ———, 1914, The habits of Astroscopus and the development of its electric organs. Carnegie Inst. Wash. Year Book No. 13, 201–203.
34. ———, 1906, A new type of electric organ in an American teleost fish, Astroscopus (Brevoort). Science, XXIII, 469.
35. ———, 1910, Origin of electricity tissues in fishes. Am. Naturalist, XLIV, 193–202.
36. ———, and C. F. Silvester, 1906, The electric organ of the star-gazer, Astroscopus (Brevoort). Anat. Anz., XXIX, 387–403.
37. Darwin, Charles, 1850, Origin of species. Pt. I, p. 234.
38. Dixon, R. Battery fish. Edison Monthly.
39. Engelmann, 1894, Die Blätterschicht der electrischen Organe von Raja in ihren genetischen Beziehungen zur quergestreiften Muskelsubstanz. Arch. f. d. ges. Physiol., LVII, 149–180.
40. Ewart, 1888, The electric organ of the skate. Phil. Trans. Roy. Soc. London, CLXXIX-B, 399, 539.
41. ———, 1892, The electric organ of the skate. Observations on the structure, relations, progressive development, and growth of the electric organ of the skate. Phil. Trans. Roy. Soc. London, CLXXXIII-B, 389–420.
42. Faraday, 1844, Experimental researches in electricity. Phil. Trans. Roy. Soc. London. 1843, 17–32.
43. Borchert, M., 1905, Ueber eine bisher unbekannte Gesetzmässigkeit im Zentralnervensystem von Torpedo. Anat. Anz., XXVI, 289–292.
44. Fritsch, G., 1886, Die äussere Haut und die Seitenorgane des Zitterwelses (Malapterurus electricus). Sitzber. d. k. preuss. Akad. d. Wiss., Phys.-math. Kl., 415–436.
45. ———, 1891, Weitere Beiträge zur Kenntniss der schwach elektrischen Fische. Sitzber. d. k. preuss. Akad. d. Wiss., Phys.-math. Kl., 941–962.
46. Fuji, K., 1912, Researches on the electrical discharge of the isolated electric organ of Astrape by means of the oscillograph. Journ. Coll. Sci. Imp. Univ. Tokyo.
47. Garten, S., 1901, Ueber rythmische elektrische Erscheinungen im quergestreiften Skelettmuskel. Abh. d. Math.-Phys. Kl. d. k. Sächs. Ges. d. Wiss., XXVI, 329–414.
48. ———, 1899, Beiträge zur Physiologie des elektrischen Organes der Zitterrochen. Abh. d. Math.-Phys. Klasse d. k. Sächs. Ges. d. Wiss., XXV, 251–366.
49. ———, 1914, Die Produktion von Elektricität. Winterstein's Handbuch der vergleichenden Physiologie, Bd. 3, part 2.
50. Gotch, Fr., 1900, The physiology of electric organs. Schäfer's Text-book of Physiology, pt. 2, 561–591.
51. ———, 1888, Experiments on curarized torpedoes. Proc. Physiol. Soc., No. 2, p. vi.

52. Gotch, Fr., and Burch, 1896, The electromotive properties of Malapterurus electricus. Phil. Trans. Roy. Soc. London, CLXXVII-B, 347–407.
53. ———, ———, 1899, Note on the electromotive force of the organ shock and the electrical resistance of the organ in Malapterurus electricus. Proc. Roy. Soc. London, LXV, 434–445.
54. ———, and J. Burdon-Sanderson, 1887, The electromotive properties of the electrical organ of Torpedo marmorata. Phil. Trans. Roy. Soc. London, CLXXVIII-B, 487–537.
55. ———, ———, 1888, Further observations on the electromotive properties of the electrical organ of Torpedo marmorata. Proc. Roy. Soc. London, XLIII, 418.
56. Henle and Koelliker, 1847, Études sur l'électricité. Organes électriques chez l'homme ou corpuscles de Pacini.
57. Hering, 1883, Ueber duBois-Reymond's Untersuchungen der secundär-elektromotorischen Erscheinungen am Muskel. Sitzber d. k. Akad. d. Wiss. Wien, LXXXVIII, Math.-naturw. Kl., 445–471.
58. Hermann, L., 1888, Untersuchungen über die Polarisation der Muskeln und Nerven. Arch. f. d. ges. Physiol., XLII, 1–83.
59. ———, 1884, Ueber sogenannte secundär-electromotorische Erscheinungen an Muskeln und Nerven. Arch. f. d. ges. Physiol., XXXIII, 103–168.
60. Herrick, C. J., 1915, An introduction to Neurology.
61. ———, 1899, The cranial and first spinal nerves of Menidia, a contribution upon the nerve components of the bony fishes. Arch. Neurol. & Psychopath., II, 21–319.
62. Hughes, J. G., Jr., 1915, A peculiar structure in the electroplax of the star-gazer, Astroscopus guttatus. Jour. Morph., XXVI, 97–108.
63. Hunter, J., 1835, "Works."
64. Jordan, D. S., 1905, A guide to the study of fishes.
65. ———, and Evermann, 1896, The fishes of North and Middle America. Bull. U. S. Nat. Mus. No. 47.
66. Koike, J., 1910, Ueber die Schlagfolge des elektrischen Organes des Zitterwelses. Zitschr. f. Biol., LIV, 431–454.
67. Kimball, 1912 College Physics.
68. Kuhne, 1888, Croonian Lecture, Ueber die Entstehung der vitalen Bewegung. Proc. Roy. Soc. London, XLIV, 427–448.
69. Loeb, J., and R. Beutner, 1913, Die Bedeutung der Lipoide für die Entstehung der bioelektrischen Potentialdifferenzen bei gewissen pflanzlichen Organen. Biochem. Zeitschr., LI, 288–299.
70. Lorenzini, 1678, Osservazioni intorno alle Torpedini.
71. Marey, 1879, Nouvelles recherches sur les poissons électriques; caractères de la décharge du gymnote, effets d'une décharge de torpille, lancée dans une téléphone. Compt. rend. Acad. d. sc., Paris, LXXXVIII, 318–321.
72. Matteucci, 1844, Traité des phenomènes électro-physiologiques des animaux.
73. Mencl, E., 1901, Contributions to the histology of the electrical centers of Torpedo marmorata. Bull. Royal Bohemian Scientific Assoc., Class Math.-Nat. Hist. No. XX.
74. Moreau, F., 1877, Mémoires de Physiologie.
75. Ogneff, 1898, Einige Bemerkungen über den Bau des schwachen electrischen Organs bei den Mormyriden. Zeitschr. f. Wiss. Zool. LXIV, 565–595.
76. Pacini, 1852, Sulla struttura intima dell' organo ellettrico del gimnoto. Gazz. med. ital. feder. toscana, 2. s. II, 305, 321, 329, 346.
77. Portier, P., 1906, Les poissons électriques. Bull. du Musée Océanographique No. 76.
78. Robin, 1847, Ann. d. sci. nat. (zool.), VII, 193–302.
79. Rollet, A., 1891, Denkschr. der math.-natur. Klasse d. k. Akad. d. Wiss. Wien, LVIII.
80. Sachs, C., 1881, Untersuchungen am Zitteraal, bearbeitet von duBois-Reymond.
81. Schäfer, 1900, Text-book of physiology.
82. Schlichter, H., 1906, Ueber den feineren Bau des schwachelektrischen Organs von Mormyrus oxyrhynchus. Ztschr. f. wiss. Zool. LXXXIV, 479–525.
83. Schönlein, K., 1894, Beobachtungen und Untersuchungen über den Schlag von Torpedo. Zeitschr. f. Biol., XXXI, 449–523; 1896, XXXIII, 408–461.
84. Shufeldt, R. W., 1907, Formidable powers of electrical fishes. Discovery, I, No. 3.

85. Schultze, M., 1858, Zur Kenntniss der elecktrischen Organe der Fische. Act. I, Malapterurus, Gymnotus. Abh. d. naturf. Ges. zu Halle, IV, 297–331.

86. Solger, B., 1902, Ueber die "intracellulären Fäden" der Ganglienzellen des elektrischen Lappens von Torpedo. Morph. Jahrb., XXXI, 104–115.

87. Stark, J., 1844, On the existence of an electrical apparatus in the flapper skate and other rays. Proc. Roy. Soc. Edinb., II, No. 25, 1–3.

88. Studnicka, F. K., 1901, Einige Bemerkungen über die feinere Struktur der Ganglienzellen aus dem Lobus electricus von Torpedo marmorata. Sitzber. d. k. Böhm. Ges. d. Wiss., Math.-nat. Cl., No. XV.

89. Silvester, C. F., 1904, The blood vascular system of the tile-fish, Lopholatilus chamæleonticeps. Bull. Bur. Fish., XXIV.

90. Wagner, 1847, Ueber den feineren Bau des elektrischen Organs im Zitterrochen. Abh. d. k. Ges. d. Wiss. Göttingen, Phys. Kl., III, 141–166.

91. Waller, A. D., 1899, Thierische Elektrizität.

92. Walsh, 1773, Phil. Trans. Roy. Soc. London.

93. Weyl, Th., 1883, Physiologische und chemische Studien an Torpedo. Arch. f. Physiol., 117–124; Suppl. 105–126.

94. Wiedersheim, ——, Handbuch der vergleichenden Anatomie der wirbellosen Tiere.

95. Winterstein, 1914. Handbuch der vergleichenden Physiologie.

EXPLANATION OF PLATES.

I. 9.5 mm. embryo, ×250. Diagrammatic anterior view of eyes to show relation of electric tissue (stippled) to eye-muscles.

R. Ex.......Rectus externus muscle.
R. Int......Rectus internus muscle.
R. Inf......Rectus inferior muscle.
R. Sup.....Rectus superior muscle.
O. Inf.......Obliquus inferior muscle.
O. Sup......Obliquus superior muscle.
Ex. C.......External carotid artery.

II. 20 mm. embryo, ×200. Diagrammatic anterior view of eyes to show increase of electric tissue (stippled) and relation of cranial nerves to eye-muscles. Roman numerals indicate cranial nerves.

III. 20 mm. embryo, ×475. Diagrammatic median view of left eye.

IV. 33 mm. embryo, ×200. Diagrammatic anterior view of eyes to show relation of nerve III to eye-muscles and to the four separate electric organs (stippled). To left is shown the electric branch of nerve III and to right the oculomotor branch.

V. Rectus internus muscle. Four stages to show gradual growth and differentiation of electric tissue on the young muscle.

Fig. 1. 4 mm. embryo. Cross-section. Electric area slightly darker than muscle area.

Fig. 2. 9.5 mm. embryo. Cross-section. Electric area increased in size and staining capacity.

Fig. 3. 14 mm. embryo. Oblique section. Electroblasts multinucleate.

Fig. 4. 20 mm. embryo. Cross-section showing only a portion of the electric area. Electroblasts vacuolated. Myoblasts striated.

El..........Electroblast, multinucleate.
M..........Myoblast.
V..........Vacuole.
Un. N.......Undifferentiated nucleus of electroblast
N...........Nucleus of myoblast.
S...........Striations.

VI. Electroplaxes. Three stages to show growth and differentiation of adult electroplax from single electroblast.

Fig. 1. 20 mm. embryo. Single electroblast. Longitudinal section to show formation of ventral papillæ from vacuoles. Nuclei undifferentiated.

El..........Electric area of electroblast.
N..........Nutritive area.
D. V........Dorsal vacuole.
V. V........Ventral vacuole.
Un Nu......Undifferentiated nucleus.
S...........Striation.
P...........Papilla.

Fig. 2. 33 mm. embryo. Longitudinal section of electroplax. Nutritive and electric areas differentiated. Striations straight and regular.

El. Nu......Electric nucleus.
N. Nu......Nutritive nucleus.

VI. **Electroplaxes.**—*Continued*

Fig. 3. Adult. Longitudinal section of portion of electroplax. Dorsal vacuoles have entirely disappeared. Striations broken and curved.

Ch. R.	Chromatic rod.	C.	Connective tissue.
Ne.	Nerve-fiber.	C. V.	Vacuole in connective tissue.
B.	Blood-vessel.		

VII. **Photomicrographs.**

Fig. 1. 20 mm. embryo, ×80. Electric area to show relation of electric to muscle tissue.

R. Ex.	Rectus externus muscle.	R. Inf.	Rectus inferior muscle
R. Su.	Rectus superior muscle.	A.	Artery.

Fig. 2. 33 mm. embryo, ×35. Electric area to show four separate electric organs in position.

Fig. 3. 80 mm. embryo, ×35. Portion of electric area. Fibers of rectus superior muscle separated by electric nerve. Overlapping of electroplaxes shown.

Fig. 4. 33 mm. embryo, ×85. Electric nerve passing through rectus superior muscle. Oculomotor nerve. Three branches of oculomotor to right.

Fig. 5. 45 mm. embryo, ×85. Electric and oculomotor branches of III nerve, immediately after leaving brain. Ganglion of V nerve below.

Fig. 6. 45 mm. embryo, ×85. Electric and oculomotor branches of III nerve passing through the foramen in the cranial wall.

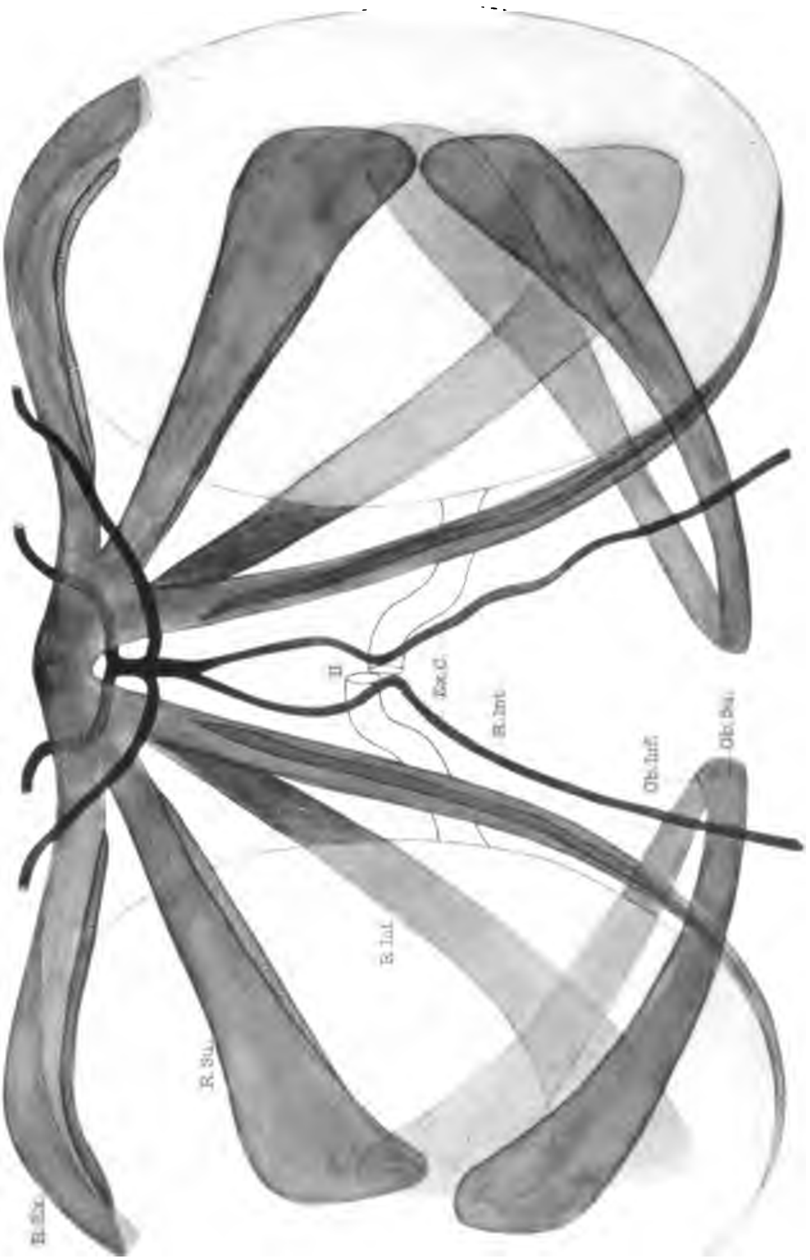
Fig. 7. 45 mm. embryo, ×85. Electric nerve in rectus superior muscle. Branches of oculomotor to right of muscle.

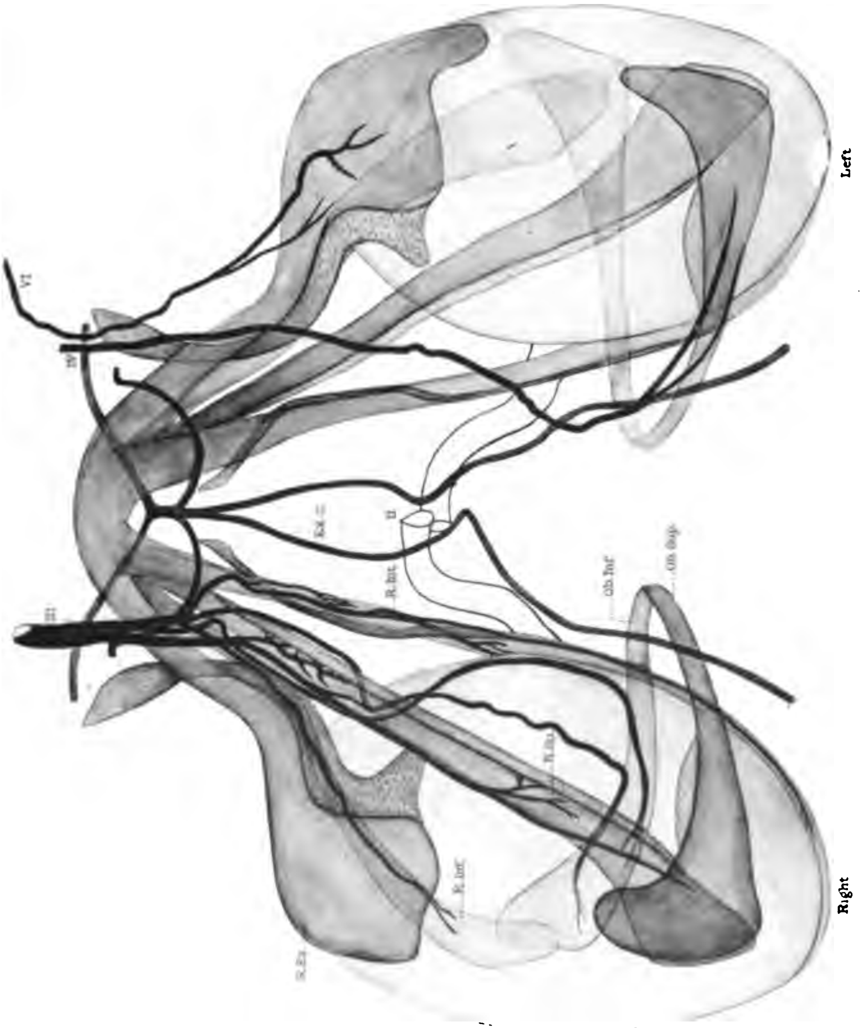
Fig. 8. 45 mm. embryo, ×85. Large branch of electric nerve entering electric organ. Oculomotor to right.

Fig. 9. 45 mm. embryo, ×85. View from opposite side of embryo. Fibers of rectus superior muscle entirely separated by electric nerve. Nerve breaking up in muscle to form branches. Oculomotor to left.

Fig. 10. 45 mm. embryo, ×85. Branches of electric nerve after leaving rectus superior muscle, going to various parts of the electric organ. Branches of oculomotor separating to go to muscles.

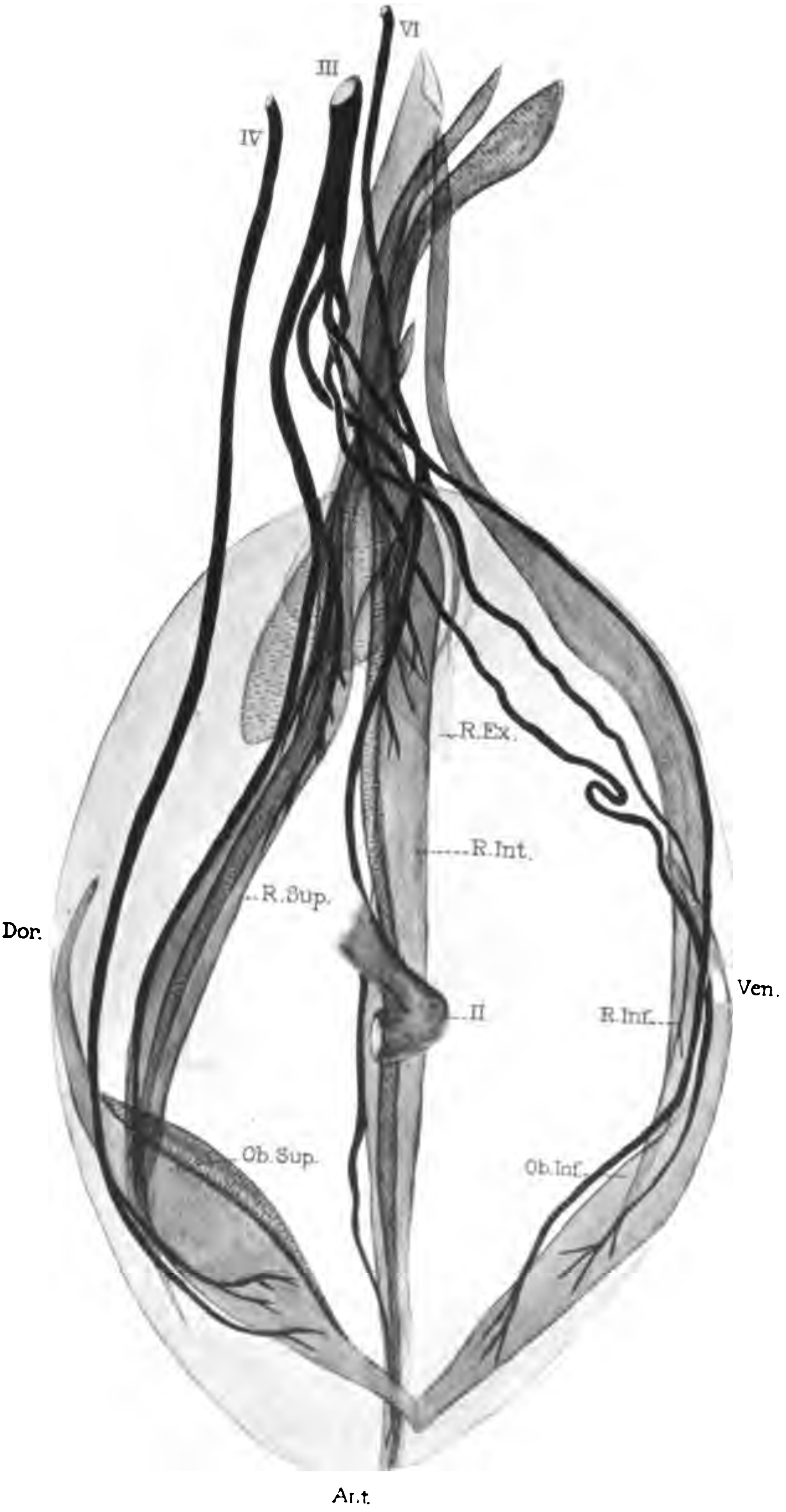
Fig. 11. 80 mm. embryo, ×85. Fibers of rectus superior muscle almost obliterated by the electric nerve.

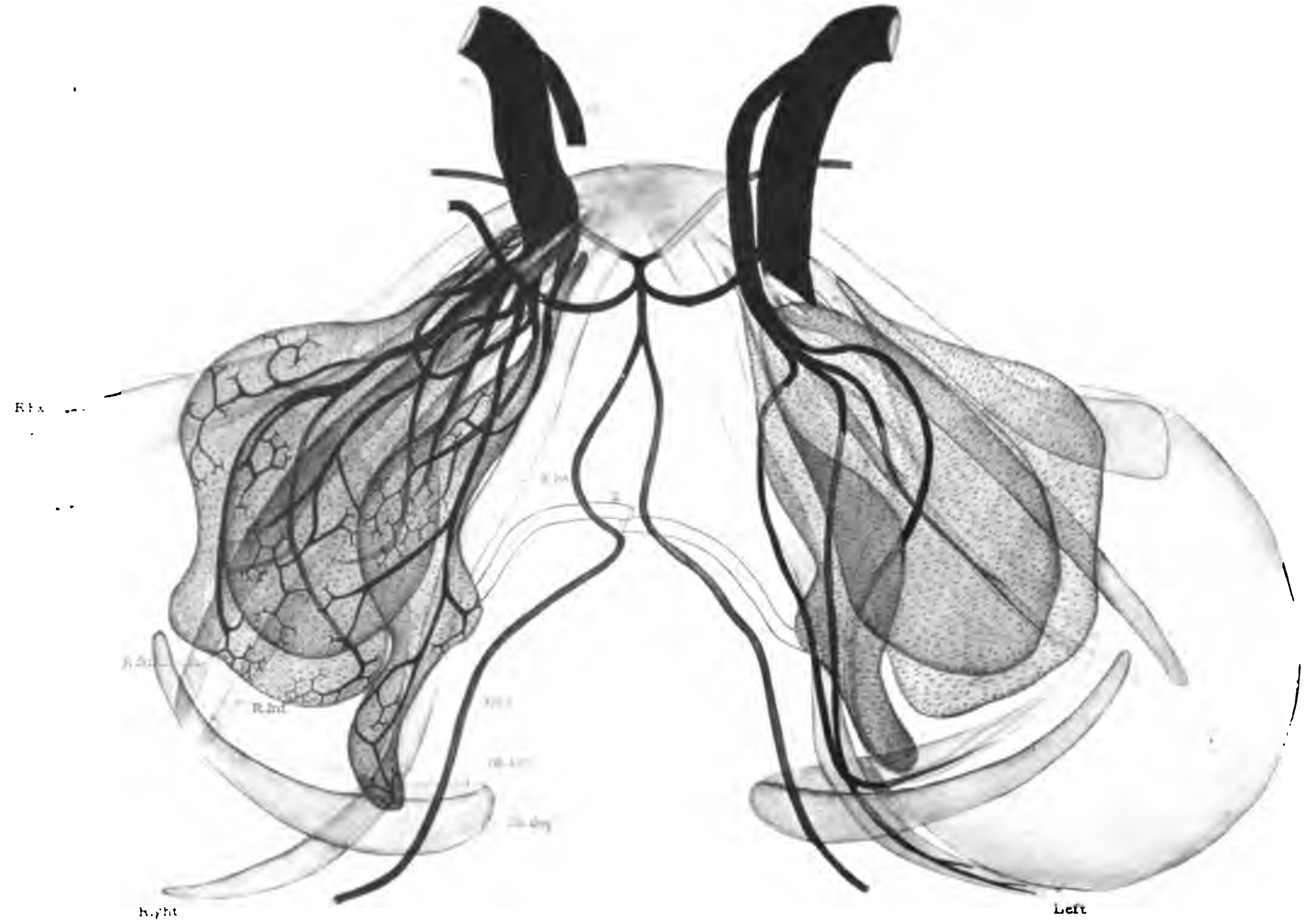

R.Ex.
R.Su.
R.Int.
II
Ext.C.
R.Int.
Ob.Inf.
Ob.Su.

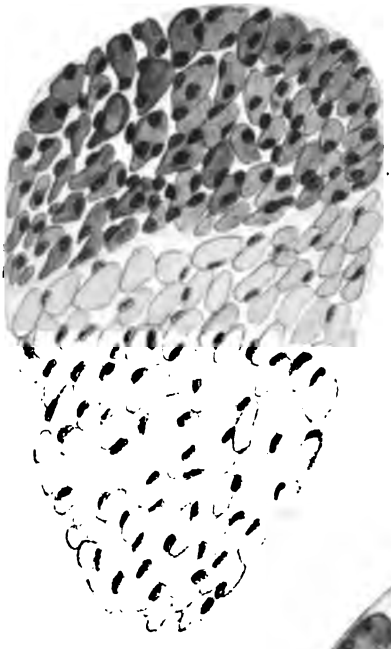

III
VI
IV
R. Ex.
Ex. II
R. Int.
II
R. Inf.
R. Su.
Ob. Inf.
Ob. Sup.
Right
Left

WHITE

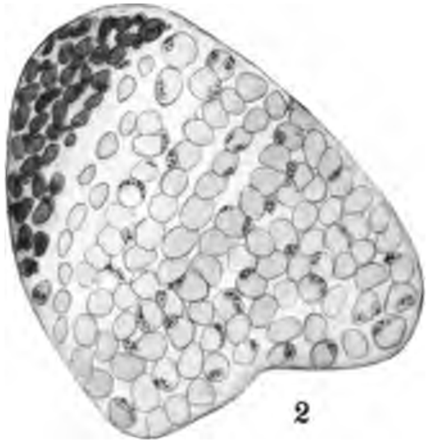
PLATE III



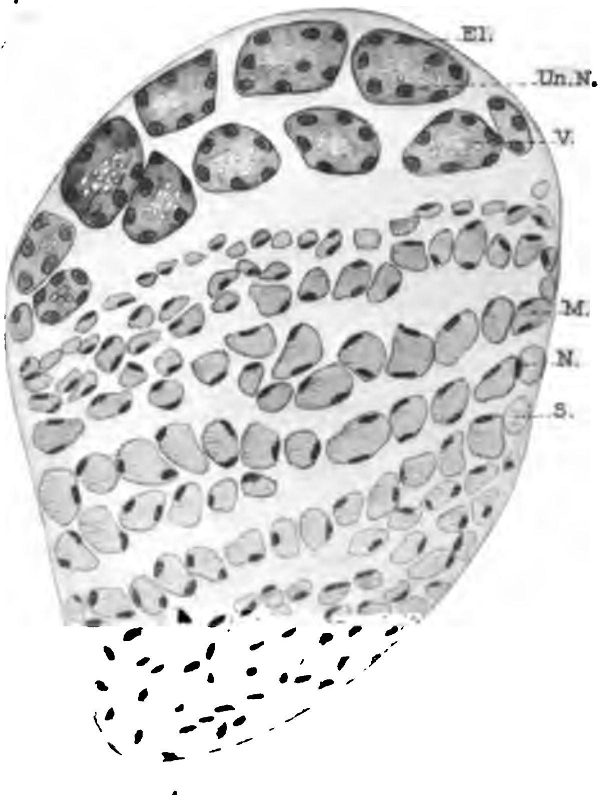

R.Int
Right
Left



3

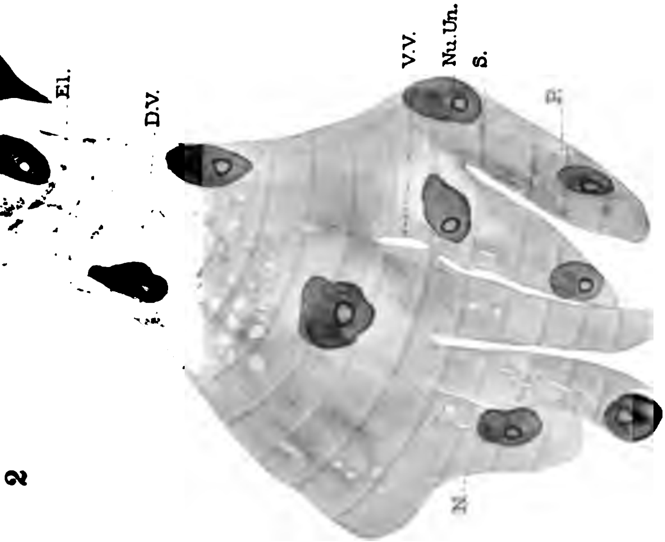


2

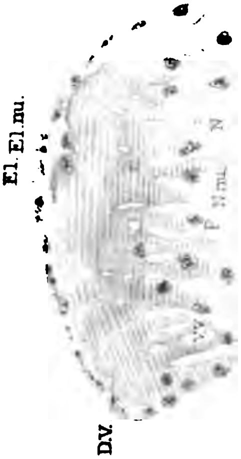


4

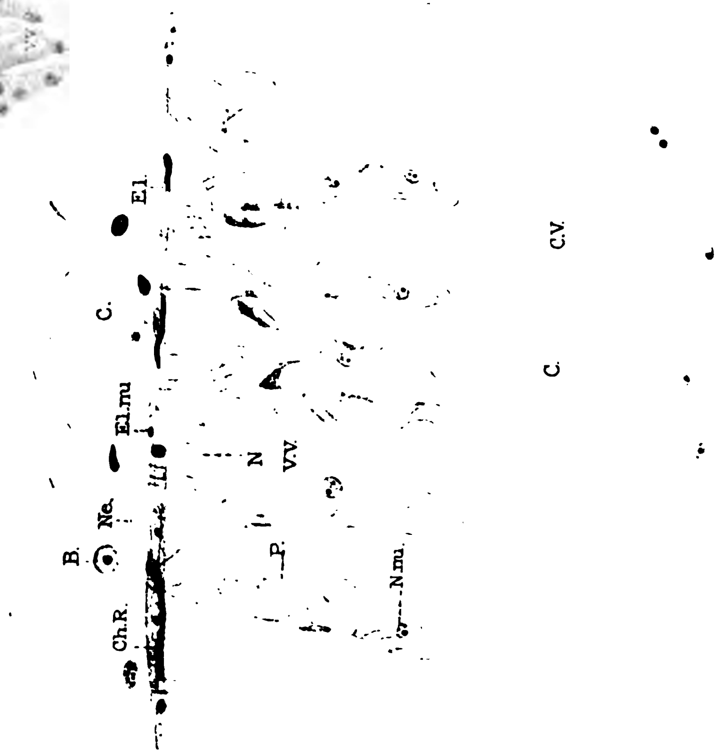
1


El.
D.V.
V.V.
Nu.Un.
S.
P.
N.

1

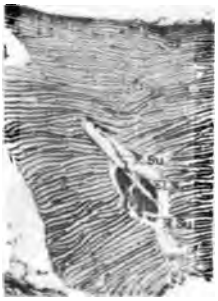

El. El.mu.
N
N.mu.
P
V.V.
D.V.

2


El.
C.
El.mu.
Li
N
V.V.
B.
Ne.
P.
Ch.R.
N.mu.
C.V.
C.

3

1



3

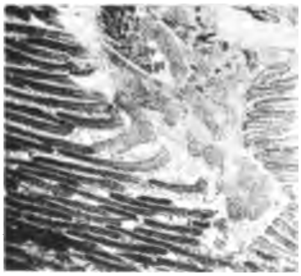
5

7

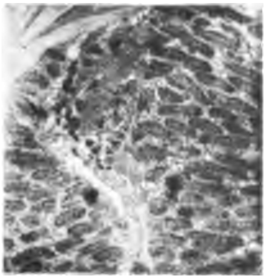
6

2

10



11



4

8

9

VII.

TOXIC EFFECTS DUE TO HIGH TEMPERATURE.

BY ALFRED GOLDSBOROUGH MAYER.

TOXIC EFFECTS DUE TO HIGH TEMPERATURE.

BY ALFRED GOLDSBOROUGH MAYER.

The experiments cited below appear to indicate that death from high temperature may be due to the accumulation of acid in the tissues.

Reef corals from Tortugas, Florida, were kept at a constant temperature in warm ocean-water for 60 minutes in a thermostat, in the dark the temperature remaining constant within about 0.1° C. throughout the hour. In this manner the temperature was found that is just sufficient to kill the coral. The results are as follows:

	°C.		°C.
Acropora muricata	34.7	Mæandra areolata	36.8
Orbicella annularis	35.6	Porites furcata	36.85
Porites astræoides	35.8	Favia fragum	37.05
Porites clavaria	36.4	Siderastrea radians	38.2

It is apparent that those corals which live in cool, relatively agitated water, free from silt, are those that can not withstand high temperatures, whereas those which live in the hot, silt-laden shallows near shore are, generally speaking, forms which can resist high temperature. *Favia fragum* is, however, an exception, for it lives not only in the shallows close to shore, but also in the pure water of the seaward reefs.

If sea-water be charged to saturation with carbon-dioxide gas from a soda-water siphon, the corals show nearly the same relative resistance to the toxic effect of carbonic acid as they do to high temperature. Thus these corals from least to most resistant are *Acropora muricata*, *Orbicella annularis*, *Porites astræoides*, *P. clavaria*, *P. furcata*, *Mæandra areolata*, *Favia fragum*, *Siderastrea radians*. *A. muricata* die in less than an hour and *S. radians* survive for more than 4 hours. In my experiments, however, *M. areolata* appeared to be slightly more resistant to CO_2 than does *Porites furcata*, whereas the reverse is the case with high temperature; but the two forms are so nearly alike in their reaction that the discrepancy may be due to errors in experimentation or to individual differences of constitution in the specimens, for there is considerable range in this respect. The toxic effect of CO_2 may, however, be independent of asphyxiation, and this supposition is strengthened by the fact that with the exception of *Acropora muricata*, all of these corals can survive in the dark for more than 11 hours in sea-water deprived of oxygen under an air-pump; and even *Acropora muricata* can withstand 6 hours of this treatment.

It will be recalled that Henze (1910) found that sea-anemones consume less oxygen as the oxygen in the surrounding sea-water is reduced; and in 1917 McClendon discovered that the medusa *Cassiopea* can live in the dark for more than 7 hours without oxygen and under this condition it produces no appreciable amount of CO_2. In other words, these cœlenterates can, if deprived of oxygen, suspend their metabolism for a more or less protracted period without apparent injury.

Moreover, I find that a constant high temperature causes death in the same time, whether the sea-water surrounding the corals has the normal concentration of about 4.3 c.c. oxygen per liter, or the oxygen be supersaturated at 6.6 c.c. per liter, or reduced by being placed under an air-pump to 1.7 c.c. per liter. Apparently, therefore, there is no direct relation between the oxygen supply and the death temperature. The death temperature also remains the same whether the corals be in sunlight or in darkness.

Using Winkler's method, tests were made upon five of the common reef corals of Tortugas to determine their relative rates of consumption of oxygen. In these experiments the corals were kept in the dark to prevent photosynthesis in their commensal plant-cells, for in sunlight the surrounding water soon becomes supersaturated with oxygen from this cause. Care was also taken to use very small specimens of the various species of corals and to place them in large glass jars hermetically sealed, each containing about 2 liters of sea-water. The corals were kept for 5 hours in these jars in darkness in a water thermostat at 28.5° C. and daily experiments were made upon each of them for 11 successive days. The polyp-bearing area of each coral was then determined by a planimeter and the relative weight of living substance per square centimeter was obtained by killing specimens of these corals in formalin, hardening in alcohol, and then dissolving away the calcareous substance in nitric acid and weighing the tissue in sea-water. Prepared in this manner a square centimeter of the animal substance of *Acropora muricata* weighs 0.032 gram; while *Orbicella annularis* weighs 0.17, *Favia fragum* 0.059, *Mæandra areolata* 0.109, and *Siderastrea radians* 0.125 gram per square centimeter.

The experiments showed that *Siderastrea radians* at 28.5° C. consumes 0.0032 c.c. of oxygen per square centimeter of its polyp-bearing surface per hour, and as each square centimeter of the fleshy substance of *S. radians* weighs 0.125 gram, each gram of animal matter of the coral consumes 0.0256 c.c. of oxygen per hour, the oxygen being measured at 760 mm. pressure and 0° C. Thus taking the behavior of *S. radians* as a standard and calling its oxygen consumption per gram of its living substance 1, the relative rates of oxygen consumption per hour in the other corals at 28.5° C. per gram of living substance of the coral are as shown in the table herewith.

Name of coral.	Relative oxygen consumption.
Siderastrea radians.	1.0
Mæandra areolata..	3.8
Favia fragum......	5.5
Orbicella annularis.	6.1
Acropora muricata.	18.7

If the rate of consumption of oxygen be taken as a measure of the metabolism of the corals, it appears that the metabolic activity bears an inverse ratio to the coral's ability to withstand the effects of carbonic acid and their ability to resist high temperature follows nearly the same law. It seems possible, therefore, that under the influence of high temperature carbonic acid may accumulate in the tissues faster than it can be eliminated, and acids being toxic would soon cause death.

In the case of the scyphomedusa *Cassiopea*, I find that for temperatures below 37° there is no time factor—that is to say, if the pulsating subumbrella ring deprived of sense-organs and having an entrapped neurogenic contraction wave be taken from sea-water of about 29° and placed in water of 36° C., it at once assumes a rate characteristic of this higher temperature, and this rate is the same as if the temperature had been slowly raised so that two or more hours elapsed before the temperature rose from 29° to 36° C. Moreover, the reaction is completely reversible, the rate returning at once to the original when the ring is replaced in sea-water at 29°. At about 37° or 38°, however, the rate of the nerve impulse begins to decline sharply, and I find that this is subject to a time factor, the decline becoming more and more pronounced as the heat, even though constant in temperature, is continued. Moreover, when the superheated ring is replaced in sea-water at 29° C., the pulsation is slower than it was before being subjected to the excessive heat. A time factor for the effect of extreme heat has been known since the time of Sachs (1865) and has been studied by Blackman (1905) and others. After about an hour, more or less complete recovery of rate may take place, especially if the heat had not been too great or exposure to its influences too long. In other words, at injuriously high temperatures CO_2 may accumulate more rapidly than the tissues can eliminate it and the time-factor, according to my hypothesis, is due to this fact.

Thus in a typical case, a nerve-wave in *Cassiopea* having a rate of 100 at 29.3° had the following rates when heated to 37.8° C.: 100 at 29.3°, 125 at 35.6°, 126 at 36.7°, 127 at 37.3°, 120 at 37.8°. Thus the nerve-conduction became more and more rapid up to 37.3° C., above which it declined sharply, becoming 120 after being for about 20 minutes at 37.8° C. The animal was then taken from 37.8° C. and replaced in sea-water at 29.3° C., and its rate declined at once to 80, thus being 20 per cent less rapid than it was at the beginning of the experiment; but after remaining in sea-water at 29.3° C. for nearly 2 hours the original rate was completely restored.

It will be recalled that Blackman (1905) and Harvey (1911) advanced the theory that some enzyme might be destroyed by the excessive heat, and, being essential to nerve-conduction, its loss caused the rate to decline. It is also possible, however, that some toxic-acid substance is formed under the influence of excessive heat, its rate of formation being commensurate with the metabolism of the tissues. It is easy to see how an acid of this sort might be eliminated and the rate gradually restored when the animal is replaced in normal sea-water, whereas if an enzyme were destroyed it might not so readily be replaced.

In any event, one or the other of the above-mentioned hypotheses seems more in accord with the facts than does Winterstein's asphyxiation theory, or the theory that death from heat is due to coagulation of proteid substances. Death occurs at too low a temperature for coagulation in most if not all proteids; and when killed the animals are fully relaxed, as shown by Harvey. Also, coagulated proteins could not readily be eliminated when the animal was restored to water at normal temperature, coagulation being a practically non-reveısible process.

SUMMARY.

It seems possible that death from high temperature may be due to the accumulation of acid (possibly H_2CO_3) in the tissues, the rate of formation of this acid being related to the rate of metabolism of the tissues. Thus animals of the same class having a high rate of metabolism, as measured by oxygen consumption, are more sensitive to heat and to CO_2 than are those having a low rate of metabolism.

LITERATURE CITED.

BLACKMAN, F. F. 1905. Annals of Botany, 19, p. 281.
HARVEY, E. N. 1911. Carnegie Inst. Wash. Pub. 132, p. 27.
HENZE, M. 1910. Biochem. Zeitschrift, 26, p. 266.
McCLENDON, J. F. 1917. Year Book Carnegie Institution of Washington for 1917.
MAYER, A. G. 1908. Carnegie Inst. Wash. Pub. 102, p. 115.
SACHS, JULIUS. 1865. Handbuch der Exper. Physiologie, p. 52.
WINKLER, L. W. 1888. *vide* Treadwell and Hall, 1905, Analytical Chemistry, 2, p. 574.
WINTERSTEIN, H. 1905. Zeitsch. für Allgemeinen Physiologie, 5, p. 323.

VIII.

NERVE-CONDUCTION IN DILUTED AND IN CONCENTRATED SEA-WATER

BY ALFRED GOLDSBOROUGH MAYER.

One text-figure.

NERVE-CONDUCTION IN DILUTED AND IN CONCENTRATED SEA-WATER.

By Alfred Goldsborough Mayer.

Ring-shaped strips of subumbrella tissue of the scyphomedusa *Cassiopea xamachana* were deprived of marginal sense-organs and placed in concentrated sea-water in order to determine the effect of concentration of electrolytes upon their rate of nerve-conduction.

These results, combined with those previously obtained[1] with diluted sea-water, are as follows:

Table I.

(Illustrated by figure 1.)

Salinity 0/00 salt in grams in 1,000 grams of sea-water.	Relative salinity, that of normal sea-water being 100.	x Relative electrical conductivity at 30° C., that of normal sea-water being 100.	Hydrogen-ion concentration of the solution in terms of P_H.	y Relative rate of nerve-conduction, that in normal sea-water being 100.	Probable error of rate.	Remarks.
18.13	0.5	54.1	8.1	54.16	0.79	Normal sea-water of 8.23 P_H diluted with distilled water of 7.9 P_H.
21.74	0.6	64.3	8.2	65.72	1.01	
25.37	0.7	71 5	8.2	73.91	1.16	
29.00	0.8	81.4	8.2	79.51	1.18	
32.61	0.9	92.2	8.2	91.44	1.00	
34.43	0.95	96.4	8.2	96.23	0.67	
36.24	1.00	100	8.22	100.	....	Normal sea-water.
39.58	1.092	107.8	8.23	101.3	0.52	Sea-water evaporated in sunlight, and its alkalinity restored to 8.1 to 8.2 P_H with HCl.
41.59	1.148	113.6	8.2	107.5	0.71	
49.18	1.357	128.7	8.13	104.3	0.95	
60.96	1.682	153.9	8.1	76.9	0.63	

The sea-water was concentrated by evaporation in sunlight, and its alkalinity reduced to that of normal sea-water by adding a slight amount of hydrochloric acid, the hydrogen-ion concentration being determined colorimetrically by comparison with a graded set of thymolsulphonephthalein tubes prepared and standardized by Professor J. F. McClendon. These readings were also checked by comparison with those of a Leeds and Northrup potentiometer.

The hydrogen-ion concentration of the sea-water at Tortugas is usually between 8.1 and 8.23 P_H, and many experiments made in 1917 showed that the rate of nerve-conduction remains practically constant and normal in sea-waters ranging from 7 to 8.65 P_H; the relatively acid water is made from normal sea-water treated with HCl and the alkaline water from normal sea-water treated with $HNaCO_3$, and Na_2CO_3.

---

[1] Mayer, A. G., 1917, Papers from Department of Marine Biology, Carnegie Inst. Wash., vol. 11, pp. 1-20.

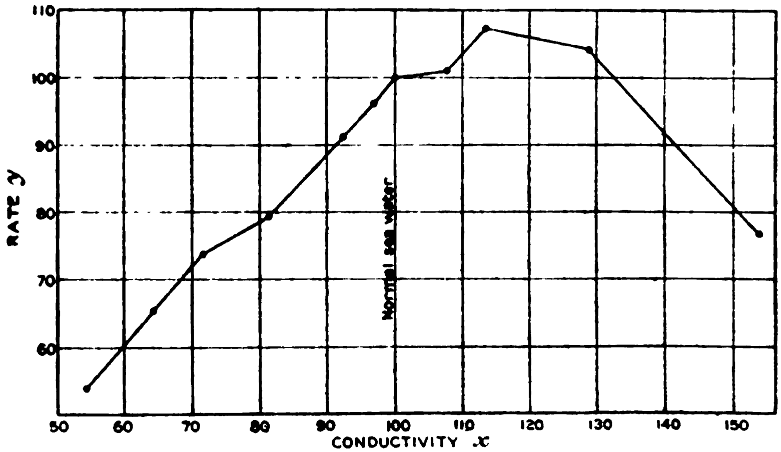


FIGURE 1.

If, however, the water is made as acid as 6.7 P_H, the rate falls slightly so as to be about 98, that in normal sea-water being 100; and at 6.3 P_H the rate declines to 80 with greatly reduced muscular activity; but it recovers in all respects almost immediately upon being replaced in normal sea-water.

At 30° C. the neutral point is about 6.8 P_H and it then appears that in sea-water any excess of $H^{\cdot}$ over OH' is decidedly depressant, while correspondingly alkaline sea-water produces no ill effects.

Assuming the electrical conductivity of normal sea-water of 36.24 0/00 salinity and 8.22 P_H to be 100, it appears from table I and figure 1 that the rate of nerve-conduction augments in practically a straight line as the relative electrical conductivity of the sea-water increases from 51 to about 108, above which the rate falls off rapidly, becoming about 77 in concentrated sea-water having an electrical conductivity of 152.9.

The formula is $y=0.945x+4.4$; y is the rate of nerve-conduction, that in natural sea-water being 100; x is the electrical conductivity of the sea-water, that of normal sea-water being 100.

The electrical conductivity of the sea-water was determined by Kohlrausch's method, using a tunable telephone and a Leeds and Northrup revolving drum bridge. The figures given in table I are for sea-water at 30° C.

It was found that the electrical conductivity of sea-water of 36.24 0/00 salinity and 8.22 P_H, at 30° C. was 4.21 times that of N/10 KCl at the same temperature. If, however, the sea-water be compared with this potassium-chloride solution, both at 25° C., the ratio of their electrical conductivities became as 1 to 4.179.

The salinity of the sea-water was determined by titration with silver nitrate, using potassium chromate as an indicator and titrating against a sample of standard sea-water from Professor Knüdsen's laboratory.

The fact that the rate of nerve-conduction increases in a straight-line ratio in comparison with the increase of electrical conductivity suggests a causal relation between the two, as was pointed out by Ralph S. Lillie, 1916,[1] but the same ratio exists between the concentration of the dissociated cations $Na^{\cdot}$, $Ca^{\cdot\cdot}$, $Mg^{\cdot\cdot}$, and $K^{\cdot}$, surrounding the nerves and the rate of nerve-conduction; and thus the rate may be dependent upon the concentration of these electrolytes rather than upon their electrical conductivity as such. Thus, in experiments made in 1917 upon *Cassiopea*, we find that if the electrical conductivity of 0.601 *m.* NaCl be taken as 1.00, that of an isotonic solution composed of 81.1 c.c. of 0.601 *m.* NaCl + 14.36 of 0.39 *m.* $MgCl_2$ is 0.932; yet if the rate in the pure sodium chloride be 100, that in the $NaCl+MgCl_2$ is 115. Thus the rate has increased while the electrical conductivity has diminished. This experiment is a striking illustration of Loeb's law that a bivalent cation tends to offset the injurious effects of a univalent cation and even magnesium, a well-known depressant, acts as a stimulant for rate of nerve-conduction in association with sodium.

Comparing the effects of these solutions with that of normal sea-water, all being practically isotonic one with the other, we find:

Rate of nerve-conduction is 100 in natural sea-water of 36.24 0/00 salinity, 8.22 P_H, and 1.00 relative electrical conductivity.

Rate of nerve-conduction is 79.6 in 0.601 *m.* NaCl of 8.2 P_H, and 0.996 relative electrical conductivity.

Rate of nerve-conduction is 92.2 in 81.1 c.c. of 0.601 *m.* NaCl+ 14.36 of 0.39 *m.* $MgCl_2$ of 8.2 P_H, and 0.94 relative electrical conductivity.

It may be of interest to see that diluted sea-water is less depressant for nerve-conduction than abnormally concentrated sea-water.

The injurious effects of concentrated sea-water upon regeneration and growth have been studied by Loeb[2] and by Goldfarb,[3] and there is a general resemblance between their curves and ours for the rate of nerve-conduction, excepting that for regeneration somewhat dilute sea-water seems to be more favorable than normal sea-water, whereas in nerve-conduction the highest rate is obtained in slightly concentrated sea-water.

---

[1] Lillie, R. S., 1916, American Journal Physiology, vol. 41, pp. 126–136.

[2] Loeb, J., 1891, Organbildung und Wachsthum, 82 pp., Würzburg.

[3] Goldfarb, A. J., 1914, Papers from the Tortugas Laboratory, Carnegie Inst. Wash. Pub. No. 183, pp. 83–94.

IX.

A STUDY OF RESPIRATION IN ALCYONARIA.

BY LEWIS R. CARY,
Department of Biology, Princeton University.

A STUDY OF RESPIRATION IN ALCYONARIA.

By Lewis R. Cary.

Although the respiration of many species of invertebrates has been studied in considerable detail, the only references to that of Alcyonaria which have come to the attention of the writer are those given by Montuori (1913), who studied two species, *Alcyomeum pallidum* and *Gorgonia cavolinii.* Two records only are given for each species, a small and a larger specimen having been compared in each case. In these experiments the total weight of the colony was taken as the basis of comparison without taking into account the proportion of inert skeletal material—the spicules in the first species and the spicules and chitinous axis in the latter.

Benedict (1915) has emphasized particularly the importance of the proportion of active protoplasmic tissue as shown even in the comparison of individuals of different sexes of the same species.

The observations herein recorded were made as part of a study of the ecological factors determining the distribution of Alcyonaria on the coral reefs of southern Florida. The data from which the amount of living tissue in the several species could be calculated were obtained three years ago in connection with a study of the importance of the alcyonaria as coral-forming organisms (Cary, 1915). A series of experiments were also carried out on the same species to determine their powers of resistance to increased temperature in order to discover whether or not there is any direct relationship between the rate of respiration and the temperature at which any species is killed; in other words, to determine whether the death of marine animals when subjected to high temperatures is in reality the result of asphyxiation, as Winterstein (1905) has maintained.

EXPERIMENTAL.

The respiration chambers consisted of museum jars, with clamped tops fitting on rubber gaskets. The capacity of the several jars varied from 960 c.c., to 1,250 c.c. as was determined by weighing each one empty and again when filled with distilled water at a known temperature. In all experiments with Alcyonaria the displacement of the specimen was determined and subtracted from the capacity of the jar. Frequently, when it was desired to make several determinations at intervals on the same specimen a fragment of coral rock was allowed to remain attached to the specimen in order to keep it upright during the period between experiments, as these organisms quickly become abnormal if allowed to lie prone on their side on the bottom (Cary, 1914, p. 86). The specimens were kept in a live-car in the intervals between the experiments, so that their condition was normal, as was shown by the fact that the colonies would remain in good condition in this live-car for at least two months.

The sea-water for each series of experiments was brought fresh from the ocean in large aquarium jars and the respiration chambers were filled by submerging them in the large jars. The temperature was controlled by placing the respiration chambers in an aquarium containing about 75 liters of sea-water. This aquarium was covered with a black box to exclude the light, as some of the species studied contained within their tissues enough symbiotic algæ (Zoöxanthellæ) to materially influence the results when the experiments were carried on in the diffuse light of the laboratory. This thermostat was connected with the running-water supply of the laboratory and during a 2-hour experiment (the usual duration) the temperature would not vary more than 0.2° C. On unusually hot days, when the temperature of the ocean-water over the shallow reef flats was subject to marked fluctuations, it was found that a much more even as well as lower temperature was obtained by cutting off the water-supply to the tank, as the early morning temperature of the water would then be maintained within half a degree throughout the day, while the variation during the time of a single experiment would not be measurable with a thermometer reading to 0.2° C.

The oxygen in the water was determined by the Winkler method, using the precautions as regards the purity of reagents described by McClendon (1917*b*). Before taking the sample for analysis at the end of an experiment the respiration chamber was inverted several times to insure complete mixing of its contents. An extended series of comparisons showed that there was no detectable difference in the results obtained by drawing the water sample—250 c.c. through a glass siphon into the empty bottle or when siphoning into a bottle by the displacement of mercury (McClendon, *l. c.*). The former, less complicated and much more rapid, method was therefore followed. The hydrogen-ion concentration (P_H) of each fresh supply of water was determined by a colorimetric method and the amount of CO_2 which it contained calculated from the P_H and reserve alkali by a conversion table (McClendon, 1917*a*). In all the experiments it was found that so great a quantity of acid other than CO_2 was being given off that the values obtained for a respiratory quotient were far higher than would be obtained from the combustion of pure carbohydrates. Since the respiratory quotients were in every instance greater than unity, whatever the duration of the experiment, they have not been included in the table.

The results of all the experiments are summarized in table 1, in which the figures in columns 2 and 3 are the averages obtained from 5 determinations on specimens of various sizes of each species. *Briareum asbestum* has a central axis composed entirely of spicules, which in macerated specimens could not be certainly separated from those formerly contained in the cœnenchyma, so that both the skeletal masses are listed under the heading spicules.

TABLE I.

Species of Alcyonaria.	Percentage of spicules.	Percentage of chitinous axial skeleton.	Percentage of living tissues.	O_2 consumed per kilogram of living tissue per hour.	Value of S/W.	Death temperature.
						°C.
Briareum asbestum..	26.66	0.0	73.34	13.99125	3.25	38.2
Eunecia crassa.....	22.66	7.90	69.44	16.735	7.75	34.5
Eunecia rousseaui...	35.60	11.28	53.12	20.75846	2.18	35.0
Plexaura flexuosa...	30.66	5.10	64.24	16.6696	6.31	35.0
Plexaura homomalla.	27.41	6.72	65.87	35.5878	5.82	35.0
Pseudoplexaura crassa	21.84	10.18	68.34	44.0062	5.90	34.5
Plexaurella dichotoma	35.86	7.57	56.57	18.585	2.72	35.0
Plexaurella sp?.....	24.95	4.45	70.63	35.7084	4.73	35.0
Gorgonia flabellum..	22.33	11.75	65.92	74.5900	9.40	37.0
Gorgonia acerosa...	19.75	7.08	73.17	54.9534	10.34	37.0
Gorgonia citrina....	35.05	5.52	59.43	61.824	9.58	37.5
Xiphigorgia anceps .	25.83	8.33	65.84	68.40	12.60	37.5

Five observations were made in each case.

DISCUSSION.

The validity of Rubner's (1885) hypothesis that heat-production (oxidation) is in direct proportion to the surface area of an organism has been denied by Montuori (1913) for all aquatic animals on the basis of his experiments recorded in that paper. On the other hand, Hoesslin (1888), Zuntz (1901), *et al.*, have maintained that their results on fishes support Rubner.

In Alcyonaria the surface in contact with the water varies greatly in different states of contraction or expansion of the colony. In the 12 species recorded in table 1 the surface area when expanded—all of them being able to completely retract their polyps—varied from 1.25 to 3.25 times that when the colony was in complete retraction. Since each species when undisturbed would arrive at complete expansion within a few moments after being put into the dark chamber, while on the other hand a slight agitation of the respiration chamber sufficed to cause them to remain contracted, the comparison of the consumption of oxygen under each of these conditions was a simple matter. While there were slight variations in the actual consumption of oxygen of any specimen in such a pair of experiments, the higher metabolism occurred as frequently when the specimen had all its polyps completely withdrawn as when they were in full expansion.

Although the area of tissue exposed to the water had no constant effect on the rate of respiration, there was a striking relationship between the proportion of surface to weight of the several species and the rapidity of their respiration. As shown in column 6 of table 1, those species which have the greatest surface for a unit of weight $\left(\frac{\text{cm}^2}{\text{gms.}}\right)$ have by far the highest metabolism. The areas used in these calculations were determined accurately for each specimen by measuring the length and the diameter of each branch of a colony and treating them as cylinders or cones according to their shape. Under the conditions necessarily imposed while making the measurements, the colonies were always in a state of complete contraction, so that, when the specimens were in full expansion, the values of S/W would be from 1.25 to 3.25 times greater than those recorded in the table.

RELATION BETWEEN DEATH TEMPERATURE AND RATE OF METABOLISM.

Column 7 of the table gives the temperatures found to be fatal to the different species on an exposure of 1 hour. When compared upon this basis the species studied fall into several groups; 2 of them succumb at 34.5° C.; another larger group finds the fatal temperature at 35° C., 2 others at 37° C., 2 at 37.5° C., while 1 species only could withstand temperatures up to 38.2° C. Although the last-mentioned species has the lowest respiration rate as well as the greatest power to withstand high temperature, there is no constant relationship between the death-temperature and the respiration-rate when all the species are compared. All of the species of the genus *Gorgonia* and the closely related *Xiphigorgia*, which have as a group the highest rate of respiration, are next to *Briareum* the most resistant to increased temperature. The two forms which are killed at the lowest temperature include *Eunecia crassa*, with next to the lowest rate of respiration, and *Pseudoplexaura crassa*, which stands eighth in the order of magnitude of respiration. Taken all together these observations offer no support to the hypothesis of Winterstein (1905), but on the contrary indicate that some other factor is the controlling agency in the ability of a marine organism to withstand high temperatures.

The heat experiments were performed in an open jar containing 6 liters of water and at the end of any experiment the amount of oxygen contained in the water was more than sufficient to allow respiration to go on in a normal manner if no other factor than oxygen tension were concerned. The acidity of the water at the close of the heat experiments was always greater than in respiration experiments carried on at 27.5° C. This may be only an expression of the abnormality of their metabolism at high temperatures, or have a causal relation to the death of the organism.

LITERATURE CITED.

For an extended list of the literature on Respiration consult Krogh, "The Respiratory Exchange in Animals and Man," 1916.

BENEDICT, F. G. 1915. Factors affecting basal metabolism. Jour. Biol. Chem., vol. XX, 263.

CARY, L. R. 1914. Observations on the ecology and growth rate of Gorgonians. Carnegie Inst. Wash. Pub. No. 182, 81.

———. 1915. The Alcyonaria as a factor in reef limestone formation. Proc. Nat. Acad. Sci., vol. I, 285.

HOESSLIN, N. VON. 1888. Über die Ursache der scheinbaren Abhängigkeit des Umsatzes von der Grosse der Körperoberfläche. Arch. f. Physiol., Bd. 2., 323.

MCCLENDON, J. F., C. C. GAULT, and S. MULHOLLAND. 1917*a*. The hydrogen-ion concentration, CO_2, tension, and CO_2 content of sea-water. Carnegie Inst. Wash. Pub. No. 251, 21.

MCCLENDON, J. F. 1917*b*. The direct and indirect calorimetry of Cassiopea xamachana: The effect of stretching on the rate of the nerve impulse. Jour. Biol. Chem., vol. XXXII, No. 2, 275.

MONTUORI, A. 1913. Les processus oxydatifs chez les animaux marins en rapport avec la loi de superfice. Arch. Ital. d. Biol., T. LIX, 213.

RUBNER, M. 1883. Ueber den Einfluss der Körpergrösse auf Stoff- und Kraftwechsel. Zeits. f. Biol. 19, 536.

WINTERSTEIN, H. 1905. Wärmelähmung und Narkose. Zeits. f. Allg. Phys., 5, 323.

ZUNST, N. 1901. Ein Respirationsapparat für Wasserthiere. Verh. d. Berliner-Physiol. Gesel., 543.

X.

MIGRATION OF INSECTS TO REBECCA SHOAL LIGHT-STATION AND THE TORTUGAS ISLANDS, WITH SPECIAL REFERENCE TO MOSQUITOES AND FLIES.

BY S. C. BALL,
Of the Massachusetts Agricultural College.

CONTENTS.

PAGE.

Introduction 195

Means by which insects may reach Rebecca Shoal Light-house 196

Opportunities for breeding of mosquitoes at Rebecca Shoal 198

Conditions at Rebecca Shoal light-station just prior to June 26 199

Methods of obtaining insects which visited the station 200

Insects taken at Rebecca Shoal between June 26 and July 18, 1917 201

Migration of the house-fly 207

Occurrence of other Diptera at Rebecca Shoal 210

Other insects noted at Rebecca Shoal 210

Evidence that Odonata and Lepidoptera migrate to Tortugas 210

Do Sarcophagidæ breed at Tortugas? 211

Summary 212

Bibliography 212

MIGRATION OF INSECTS TO REBECCA SHOAL LIGHT-STATION AND THE TORTUGAS ISLANDS, WITH SPECIAL REFERENCE TO MOSQUITOES AND FLIES.

By S. C. Ball.

INTRODUCTION.

For the purpose of determining whether mosquitoes can, either by their own powers of flight or with the aid of favorable winds, migrate across the sea for considerable distances, the writer spent the period from June 26 to July 18, 1917, upon the Rebecca Shoal light-station. The work was undertaken under the auspices of the Carnegie Institution of Washington, Hon. G. R. Putnam, Commissioner of Lighthouses, having kindly granted permission to study at Rebecca Shoal lighthouse. Moreover Dr. L. O. Howard has kindly made timely suggestions respecting classification, but he is in no sense responsible for statements expressed herein. Except Stegomyia, the names of mosquitoes used are from Howard, Dyar and Knab, 1917.

The circumstances which suggested the desirability of such investigations were the repeated experiences of Dr. Mayer and other scientists at Tortugas, Florida, in connection with the occurrence there of mosquitoes. It appeared that these insects were abundant on Loggerhead Key only after northerly winds of several hours' duration—in other words, under conditions favorable to their migration from the mainland of Florida. Owing to the care which would be necessary in order to prevent absolutely the breeding of mosquitoes on the several keys of the Tortugas Group, particularly at Fort Jefferson on Garden Key, it was thought advisable to make observations at the nearest point to Tortugas where this difficulty could be overcome.

Rebecca Shoal light-station was chosen (1) because of its isolation from the mainland and other keys, and (2) because of its freedom from all except easily controllable breeding-places for mosquitoes. The lighthouse stands in 12 feet of water upon Rebecca Shoal, a small and entirely submerged part of the Florida Reef, 12 miles east of the Dry Tortugas and 48 miles west of Key West. The nearest point upon the mainland of Florida is Cape Sable, 105 miles northeast. Havana lies 95 miles to the south on the Cuban coast, while Cape San Antonio, at the western end of Cuba, bears southwest from Rebecca Shoal at a distance of 230 miles. Cardenas Bay lies 135 miles to the southeast.

Between Rebecca Shoal and Key West several keys of the Florida Reef intervene. Most important in relation to insect migration is the

Marquesas group. These keys are at the same time the largest and the nearest to Rebecca Shoal—24 miles east—and are largely covered with mangrove swamps which furnish breeding-places for myriads of mosquitoes. Their relation to the occurrence of these insects at the light-station will be considered later. In succession, passing eastward, lie Boca Grande, 6 miles distant from Marquesas; Man Key, Woman Key, Crawfish Key; then Mullet, Barracouta, Cottrell, and Snipe Keys at the entrance of Key West Harbor. It thus appears that any insect which reaches Rebecca light-station by flight alone must cover either 12 miles from Tortugas on the west or 24 miles from Marquesas on the east, or at least 105 miles from the mainland on the east and north, or 90 miles or more from Cuba.

The station consists of a wooden dwelling 25 feet square and 26 feet high, supported 34 feet above mean water-level upon an iron frame understructure. A 10-foot platform surrounds the base of the dwelling on all sides. The first story contains a single room 6 feet in height, which is occupied by four large wooden tanks for the storing of rain-water, and by a paint-closet and work-bench. The keeper's room, a kitchen, and pantry open into a small hall on the second floor. From here a stairway leads to the third floor, which is divided into a lamp and oil room, two assistants' rooms, and a hall. A ladder gives access to the lantern, light, and its mechanism at the top of the station. The lantern is surrounded by a narrow balcony. The diagonals of the station run north and south and east and west. At a level 8 feet above the water are two small boat-landings, one each on the southeast and northwest sides.

MEANS BY WHICH INSECTS MAY REACH THE STATION.

There are four conceivable ways in which insects may reach Rebecca Shoal light-station. First by boat, for many are brought in provisions and other stores. Several species of larder pests, including two weevils and a flour beetle, two species of moths, one in flour and another in raisins, and an occasional fruit-fly, were observed on the station. The light-house power-boats usually bring on each trip a few house-flies and occasionally a blow-fly. In one instance a *Tabanus* was introduced.

It is extremely improbable that insects are attracted to the station from vessels which must pass through Rebecca Channel at a distance of at least 0.5 mile. Indeed none of the half dozen house-flies left the yacht *Anton Dohrn* on her voyage from Tortugas to Key West on July 30, although she passed within 100 yards of the Rebecca station. It is inconceivable that flies or mosquitoes voluntarily leave the solid support of a vessel where food is close at hand and its odors strong in order to land on a small light-station at a considerable distance. Anyone who has been on board ship at sea has probably observed how closely flies, beetles, dragon-flies, and moths keep to the vessel.

A second way by which insects may reach Rebecca Shoal light-station is through the agency of birds. Several species, including the man-o'-war bird, *Fregata aquila;* noddy, *Anoüs stolidus;* sooty tern, *Sterna fuscata;* and the royal tern, *Sterna maxima,* occasionally light upon the structure. It must be said, however, that no avian parasites were found at the station.

More important is a third method, the voluntary migration of insects from the mainland or from islands. It is conceivable that during calm weather such strong fliers as the Odonata, with certain Lepidoptera and Diptera, should voluntarily leave the Marquesas or Tortugas Keys and fly in a direction which would bring them within sight or smell of Rebecca Station.

Finally, there is the wind as an agent for the distribution of insects. It is well known that strong winds blow many Lepidoptera, Hymenoptera, Hemiptera, and Diptera out over large bodies of water, such as the Great Lakes and seas. That large numbers of these fall into the water and drown is evidenced by the masses of them washed upon the shores by the waves; but it is not known what proportion of the insects survive to reach other shores.

A priori, one would expect that the strong-flying species, such as the bees and wasps among the Hymenoptera, Sphingidæ of the Lepidoptera, and Tabanidæ among the Diptera, if driven by a high wind beyond sensing distance of land, would be more capable of sustaining themselves in the air and so of being carried to safety than the heavy-bodied, weak-flying insects like the Orthoptera and the flitting Lepidoptera. The habits of the latter accustom them to short flights and considerable periods of rest. Of mosquitoes, it must be remembered that, although they are comparatively small and not as a rule called on to fly long distances in search of food, still they are light in weight, have a relatively large wing surface, and are capable of sustaining themselves in the air for long periods. The same is true of other small Diptera, particularly the gnats.

Several long migrations of mosquitos have been recorded. In his book, "Mosquitoes," Dr. L. O. Howard cites two remarkable flights which were described in a letter to him from J. D. Mitchell, of Victoria, Texas. In October 1879, during a strong east wind which had been blowing for about 3 days, an immense swarm of mosquitoes migrated in a line 3 miles wide and 50 feet high from a marsh 35 miles to the eastward; 5 days were required for the passage. In 1886 a second swarm traveled 50 miles along the west shore of Matagorda Bay in such numbers that "they clouded the sky, bent down the grass with their weight, and made all driftwood and ground the same color."

Among the most important facts established by Dr. J. B. Smith and his assistants during their investigations of the mosquitoes of New Jersey in 1902–1904 were the length and frequency of the migrations undertaken by the three species, *Aëdes sollicitans*, *A. cantator*, and *A. tæniorhynchus*. Breeding only in the salt marshes along the seashore, swarms of these three species migrate inland for distances of more than 30 miles; although capable of making these flights in calm weather, favorable sea breezes were found to hasten them.

As stated above, Dr. Mayer and others have noted in previous years that mosquitoes were abundant on Loggerhead Key only after the wind had been blowing from a certain quarter, usually the northeast or north, for a considerable period. Furthermore, a change of the wind into the east or west was always followed by a marked falling off in the numbers of mosquitoes encountered about the laboratory. The inference was that the northerly wind had blown large swarms of the insects away from the west coast of Florida and that part of them had reached Tortugas. If this inference were correct, even a small building, such as the Rebecca Shoal light-station, lying out in the ocean 18 miles east of Loggerhead, should form a sufficiently large support to attract a few mosquitoes which might be carried into its immediate vicinity. Even during a wind of such strength that the insects could not retain a foothold upon the windward side of the station, they would be able to do so under its lee.

OPPORTUNITIES FOR BREEDING OF MOSQUITOES AT REBECCA SHOAL.

Obviously, in order to draw correct conclusions concerning the occurrence of mosquitoes and flies at an isolated point, it is essential that breeding-places there be under control. It was found that the only fresh water available to mosquitoes at the Rebecca Shoal light-station was contained in three sorts of receptacles; first, the four tanks on the lower floor for the storage of water obtained by draining through iron spouts the rain-water falling on the slate roof; second, the small depressions formed in the angles of the iron frame understructure; and third, the bilge-water contained in the two boats suspended from davits.

Concerning the small pockets and crevices in the steel frame of the structure, it is only necessary to state that all water which lodged therein was entirely evaporated by the sun in less time than would be required merely for the hatching of mosquito eggs. Since rains were infrequent in the period during which these observations were made, the possibility of a replenishment was precluded. It is conceivable that in a period of daily rainfall eggs deposited by mosquitoes in these depressions might develop and by good fortune yield a few adults. Still the rapid overflow during the characteristically heavy showers would doubtless wash out most, if not all, eggs and larvæ had they been present.

The storage tanks were accessible to mosquitoes since their covers did not fit closely; therefore it was necessary to be certain that no larvæ were present. Careful examination of the surface of the water and of the contents on June 27 failed to reveal a single trace of larvæ, pupæ, molted skins, or adults which might have been drowned in the act of depositing eggs. Indeed, the lighthouse keepers, Mr. Lopez and his assistants, all of whom were familiar with the larvæ, agreed that they had never noted in water drawn from the tanks a single "wiggle-tail." Had larvæ been present they would probably have been detected, for the water is all drawn from the bottom of the reservoirs.

Owing to the ease and thoroughness with which frequent examinations of these tanks could be made, it was deemed unnecessary to treat the water surfaces with kerosene. Vigilance and daily examinations throughout the period of observations disclosed no sign of mosquitoes at any stage of their life-history in the reservoirs.

The third possible breeding-place (the boats), is also an extremely unfavorable one. In the case of the power launch a heavy film of lubricating oil is always present, while the sea-water in the bottom of the small dory, besides being too saline owing to evaporation, is also easily examined. No mosquitoes bred there.

From the above statement of conditions and the past experience of the keepers it is clear that mosquitoes rarely, if ever, breed at Rebecca Shoal light-station. Certainly none attempted to do so during the period covered by these investigations.

CONDITIONS JUST PRIOR TO JUNE 26.

In order to account properly for the presence of certain insects found upon Rebecca shoal light-station on June 26 it is desirable to acquaint the reader with the conditions obtaining there in the period just preceding. From the lighthouse journal it was learned that on June 13 the keepers began painting the station, working that day upon the southeast side. At that time the wind was blowing freshly from the northeast. In other words, the air-current passed swiftly across the painted surface of the building; it was not on the lee side. Following several hours of calm on June 14 a light north wind sprang up and continued until June 15, when it became somewhat westerly, finally dying out, to be followed by 36 hours of calm.

It is of interest to note than on June 15 a coat of white paint was applied to the southwest side of the dwelling, and particularly that 100 or more small gnats were later found stuck in this paint. Now, it will be seen that this southwest face of the building was, in the period following the application of the paint, in the lee of the north and northwest winds; therefore, the insects seeking a resting-place on this surface were caught. Since they are not species that would have been brought in stores, it seems a fair conclusion that they were carried by the northerly wind from the coast of Florida. The possibility is not excluded that these gnats were brought to the station on the northeast breeze of June 13 and harbored there until the application of the paint.

On June 17, following the 36 hours of calm, a light northeast wind held until June 18, when it became north-northeast. During June 19 its direction was from the east. After a day's calm, on June 21, a light breeze blew from the northeast, while that side of the dwelling was being painted. Here a single insect was later found in the paint, a strong-flying damsel-fly which may have come down the wind and been able to make a landing, even though the air-current were sheering off across the surface painted.

An examination of the station on June 26 and 27 disclosed 6 living and 1 dead house-flies, *Musca domestica*, in the lower rooms; one mutilated damsel-fly and one pentatomid on the balcony beneath and outside the lantern, and three dead gnats on the sills of the storm panes inside. The gnats no doubt ascended from the dwelling below and had been unable to find their way out. In addition to these—which, in all probability, reached the station by flight—many larder pests were discovered, including two species of moths, one breeding in stale wheat flour and the other in packages of raisins, bean weevils (*Bruchus obtectus*) in dried beans, and a heavy infestation of weevils in two cases of the pilot bread which the keepers are required to keep on hand as a provision against want during possible prolonged periods of inability to secure their usual provisions from Key West. Each biscuit contained upward of two dozen larvæ and adults.

Upon inquiry of the keepers it appeared that on June 19 "one or two mosquitoes" had been observed by them in their living quarters, but that this was a rare occurrence. The wind on the two preceding days had been from the northeast and north-northeast, so that the source may well have been the coast of Florida. It is probable that had a careful lookout been kept at that time several other specimens would have been noted.

METHODS OF OBTAINING INSECTS WHICH VISITED THE STATION.

On June 26 an insect net 5 feet in diameter and 6 feet deep was so suspended from the balcony railing beneath the lantern that any wind from the northeast, east, or southeast would spread it; but the hope that insects might be caught in this net was not realized during the entire period of observations. The lamp at this station is apparently not of sufficient strength and its flashes not of sufficient length to attract many of the insects which may be passing. During a revolution of the lamp and lenses lasting 30 seconds two groups of three similar flashes occur, each group requiring 9 seconds and being followed by a blank interval of 6 seconds.

Practically all mosquitoes and flies were taken with a hand net. In order to catch all of the former a close examination of the entire station was made several times a day in order to dislodge any which had found resting-places in dark corners. However, most of the mosquitoes were taken in the act of biting some person. During a flight it was necessary only to station one's self quietly in the windward door of a room in order to entice any mosquito present within reach of the net. After it had been observed that these insects occasionally were attracted to the corks of bottles of raspberry shrub, dishes containing some of the liquid were set in several likely positions. Examination now and then resulted in the capture of a mosquito. After dark a flash-light was of great service in locating mosquitoes which were attempting to bite.

In order to capture house-flies it was necessary only to keep a close watch in the kitchen and living rooms and to net those which approached men at work outside. It was found possible to keep the station practically free of *Mucsa domestica* in this way. During periods of continued easterly winds the larger number of those taken on a given day were captured easily just after daybreak. These had doubtless arrived during the night. Single specimens would then be noted at long intervals during the day. Thus when dusk fell the station would again be practically free from flies.

It should be stated in this connection that there were absolutely no breeding-places for *Musca domestica* upon the station. The sewage and garbage were at once committed to the sea.

INSECTS TAKEN AT REBECCA SHOAL FROM JUNE 26 TO JULY 18.

Those insects which migrated to Rebecca Shoal light-station proved to belong to four orders, Odonata, Neuroptera, Lepidoptera, and Diptera. Of the first order only two appeared, one being a large dragonfly and the other a damsel-fly. A single Neuropteran, the golden-eyed lace-wing *Chrysopa*, was taken, while the only Lepidopteran was a moth, *Syntomeida epilais* Walker. The Diptera, as anticipated, were more numerous both as to species and individuals, and it is with these that the remainder of the paper will be mainly concerned.

On the next page is a table showing the weather conditions and the kinds and numbers of insects taken.

Considering first the occurrence of mosquitoes, the table on the following page shows that during all the time from June 26 to July 3, while the wind was blowing from the east or changing into the north, only a single mosquito was noted. This was taken on July 1 at 9 p. m., and was of the species *Aëdes niger*. It is probable that its point of departure was the Marquesas Keys, 24 miles to the east. A few others appeared later on east winds.

On July 2 the wind shifted into east-northeast and continued lightly from that direction during the night. At 5 a. m. of July 3 it was north, having shifted considerably during the night; at 10 a. m. it was northeast, at 12 noon it was north and continued so during the rest of the day, freshening toward night. The weather was now clear and the moon full. No mosquitoes were noted during the day.

At $5^{h}30^{m}$ on the morning of July 4 the wind was still in the north and continued so during the forenoon. Throughout the afternoon it blew from a few points west of north, again freshening toward night. At 8 a. m. the first specimen of what proved to be one of the two important mosquito migrations appeared at the station. This arrival, as may be calculated, was at least 27 hours after the wind had begun blowing from the northeast or north, but no more were noted until between 6 and $7^{h}30^{m}$ p. m., when 3 were taken, indicating that mosquitoes in the vicinity were increasing in number.

The wind was still blowing moderately from the north at $4^{h}30^{m}$ a. m. on July 5. That mosquitoes were still about was proved by the cap-

TABLE 1.—*Insects taken at Rebecca Shoal light-station and the conditions of their occurrence.*

Date.	Winds.		Weather.	Mosquitoes.	Musca domestica.	Other insects.
	Direction.	Strength.				
June 26	E.	Fresh....	Squalls...	0	6	
27	E.	Moderate.	Do. ...	0	2	One green blow-fly, *Lucilia*.
28	E.	Do. ..	Do. ...	0	0	
29	E.	Do. ..	Do. ...	0	0	One *Tabanus*.
30	E.	Light....	Clear....	0	0	
July 1	E.	Do.	Do.	1	0	One small gnat.
2	ENE.	Do.	Do.	0	0	
3 $5^{h}00^{m}$ a. m.	N.					
$8^{h}00^{m}$ a. m.	NE.	Fresh....	Do.	0	0	
$12^{h}00^{m}$ m.	N.					
4 $5^{h}00^{m}$ a. m.	N.	Light....				
$4^{h}00^{m}$ p. m.	N. by W.	Fresh....	Do.	4	0	
$9^{h}00^{m}$ p. m.	N. by W.	Do.				
5 $4^{h}00^{m}$ a. m.	N.	Moderate.				
$5^{h}00^{m}$ p. m.	ENE.	Fresh....	Do.	21	0	One dragon-fly.
$10^{h}00^{m}$ p. m.	E. by N.					
6 $5^{h}00^{m}$ a. m.	E. by N.	Moderate.				
$8^{h}00^{m}$ a. m.	ESE.	Do. ..	Do.	2	25	
$4^{h}00^{m}$ p. m.	NE.	Light....				
$8^{h}00^{m}$ p. m.	E. by N.	Fresh....				
7 $5^{h}00^{m}$ a. m.	E by S.	Fresh....	Cloudy...			
$9^{h}00^{m}$ p. m.	E.	Light....	Clear....	0	5	One *Tabanus*.
8 $5^{h}00^{m}$ a. m.	E.	Do.	Do.	4	5	One blow-fly, *Lucilia*.
$12^{h}00^{m}$ m.	ENE.	Do.				One blow-fly, *Lucilia*.
$8^{h}00^{m}$ p. m.	E.	Do.				One neuropteran, *Chrysopa*.
9 $2^{h}00^{m}$ a. m.	E.	Do.				
$8^{h}00^{m}$ a. m.	NE.	Do.	Do.	6	3	One blow-fly, *Lucilia*.
$9^{h}00^{m}$ p. m.	NE.	Moderate.				
10 $5^{h}00^{m}$ a. m.	E.	Light....				
$4^{h}00^{m}$ p. m.	None.	Calm....		0	0	One blow-fly, *Lucilia*.
$8^{h}30^{m}$ p. m.	SE.	Strong...	Squalls...			
11 $5^{h}00^{m}$ a. m.	WSW.	Fresh....	Cloudy...	37	18	
$8^{h}00^{m}$ a. m.	SW.	Very light.				
$9^{h}00^{m}$ p. m.	SW.	Do.				
12 $5^{h}00^{m}$ a. m.	ESE.	Fresh....	Clear....	7	5	Moth, *Syntomeida*.
$8^{h}00^{m}$ p. m.	ESE.	Strong...				
13	E. by S.	Do. ...	Do.	1	1	
14	E.	Do. ...	Do.	0	2	
15	E.	Do. ...	Do.	0	1	
16	E.	Do. ...	Do.	0	0	
17	E.	Do. ...	Do.	0	1	
18	E.	Do. ...	Do.	0	0	

ture of a specimen at this early hour, just as day was breaking. Another was taken at 5^h30^m, and during the next 3 hours of the morning mosquitoes were so numerous that at times 2 or more could be captured with one sweep of the net; at 8^h15^m a. m. the count stood at 14 specimens. At intervals during the day others appeared, the last at 8 p. m., which made the total 21 for the day.

Now this marked increase in the number of mosquitoes encountered at the station, following a shift of 90° or more in the direction of the wind, from east to north by west, gains more significance as we note the interval of time elapsing between the inauguration of this change and the appearance of the insects. As already stated, at least 27 hours had passed from the time when, on the night of July 2 and 3, the wind was blowing from the vicinity of Cape Sable, 105 miles to the northeast on the southwest point of Florida, and the arrival of the first mosquito at Rebecca Shoal at 8 a. m. on July 4. That only 1 specimen was taken at this time indicates that comparatively few mosquitoes were blown out to sea from the southern capes. Not until toward night, after the wind had been blowing for over 37 hours, did the numbers begin to rise. The inference is that as the wind shifted—assuming that its direction held over the entire distance—points farther and farther north on the west coast of Florida contributed to the forced migration of mosquitoes. As will appear, the species taking part were only such as breed in salt marshes; therefore, those regions having such swamps would send out large swarms.

It will be seen that the body of the swarm encountered at Rebecca Shoal arrived on July 5, after the wind had been blowing from due north—the direction of Tampa Bay—for 48 hours or more. Obviously it can not be determined just how far up the coast these mosquitoes originated, but it is certainly possible that some of them may have come from points 150 miles to the north.

The specimens taken on this migration proved on determination to belong to two species. A single one of *Aëdes sollicitans* was captured on July 4. The other three, as well as the 21 taken on July 5, were of the species *Aëdes niger*. *A. sollicitans* and *A. tæniorhynchus* (a species closely allied to *A. niger*) have become noted for their migratory habits through the researches of Dr. Smith, of New Jersey. Breeding in the salt marshes along the seashore each summer develops immense swarms which spread inland to harass the inhabitants of cities even 30 miles away. In New Jersey *A. sollicitans* with *A. cantator* is the dominant species, *tæniorhynchus* being much less important, according to Dr. Smith:

> "*Culex tæniorhynchus* develops with *cantator* and *sollicitans* under the same conditions and migrates with them; but it does not fly so far and is always so much less abundant that it needs no special account here."

In the south, however, *A. niger* appears to be the dominant species and certainly flies, or is carried by winds, over the sea for long distances.

Late in the afternoon of July 5 the wind at Rebecca Shoal began to shift eastward, being east-northeast at 5 p. m. and east by north at 9^h45^m, blowing freshly. The last mosquito was taken at 8 p. m. Daylight of July 6 still found the wind east by north and moderate. Two mosquitoes were captured, one at 5 a. m. and the second at 8^h27^m a. m., the last for the day. These individuals were, no doubt, the rear guard of those brought from Florida by the winds of the day preceding.

No more mosquitoes appeared until July 8, when 4 were taken during the forenoon; July 9 yielded 6 more, all *Aëdes niger*. Since the wind on July 7–8 and forenoon of July 9 was east—that is, it was blowing directly along the reef from Marquesas to Rebecca Shoal—the inference is that the mosquitoes were carried by the light breeze from these islands 24 miles away. Although there are extensive brackish swamps on the Marquesas Keys, it was found, when we visited them on July 30, that most of the water had evaporated, leaving only heavy mud. Rains had been infrequent during the preceding weeks with the result that swamps, which during the rainy season give rise to multitudes of mosquitoes, were during July unproductive. There were many adult *Aëdes niger* in the grass and among the mangrove trees, however, and it was no doubt from their ranks that the few brought on east winds to Rebecca Shoal were derived.

It was hoped that another north wind would arise, so that the striking migration observed on July 4 and 5 might be duplicated. While this did not happen, I was fortunate enough to be on the station during a period of southerly breezes which brought an even more marked flight of mosquitoes. After several hours of calm on July 10, southeast squalls blew up late in the evening, lasting for two hours. The wind then veered into the south and finally held west-southwest, blowing freshly all night. At 5^h30^m a. m. of July 11 the first mosquito was captured; 32 more were added to the collection during the day, and were well distributed as to time, 2 being caught at 7^h30^m a. m. and 1 at each of the following: 8^h15^m, 8^h30^m, 8^h52^m, 8^h56^m, 8^h57^m, 9^h07^m, 9^h08^m, 9^h11^m, 9^h16^m, 10^h11^m, 10^h24^m, 11^h38^m, 11^h40^m, 12^h05^m, 12^h10^m, 12^h13^m, 12^h15^m, 1^h20^m, 1^h22^m, 1^h25^m, 3^h30^m, 4^h10^m, and 5^h09^m; between 6^h30^m and 7^h30^m p. m. 7 more were taken, but none was noted after darkness fell.

The greater number rose only to the platform level, but 8 reached the second story above, and 1 was caught by one of the assistant keepers while painting the railing above the lantern at the very top of the station, 65 feet above the water. In order to determine whether the insects were numerous just above the surface of the sea, I stationed myself on the boat-landing 8 feet from the water. One or two specimens were caught here attempting to bite, but they seemed no more abundant than at the level of the platform 30 feet above.

During the night of July 11–12 the wind veered again toward the east; at 5 a. m. July 12 it was blowing freshly from east-southeast; 7 mosquitoes appeared between 9 a. m. and 2^h20^m p. m.

Judging from the direction of the wind previous to and during this migration of July 11 and 12, we conclude that these mosquitoes were carried from the shores of Cuba. The time required for the passage was apparently less than in the earlier migration from Florida; at the end of only 10 hours after the wind, as noted at Rebecca Shoal, began to blow from the southeast—8 p. m. to 5^h30^m the following morning—the first mosquito appeared, whereas in the first case 27 hours were required. Since the strength of the wind was similar in the two cases this disparity in the time intervals may indicate that, while the second flight may have left the Cuban coast at the point nearest to Rebecca Shoal—95 miles directly south—the first swarm probably took its departure far up the west coast of Florida; for the nearest point, Cape Sable, is only 105 miles from Rebecca.

The fact that the great majority of mosquitoes taken belong to the single species *Aëdes niger* is evidence of its wide distribution and common occurrence in the south. Furthermore, this distribution is doubtless the result of the ability of these insects to sustain themselves in the air and so to be carried long distances across the sea by winds. Howard, Dyar, and Knab give the range of this species as the Antilles and Florida from Tampa southward.

On July 13 the wind, east by south early in the morning, finally settled into a strong and steady blow from due east and continued so through the next 6 days. A single mosquito taken on the morning of July 13 was the last which appeared during my stay at the lighthouse.

As Smith has shown, mosquitoes do not rise from the ground while strong winds are blowing. Had any been carried to the station it is improbable that they could have made a landing even on the lee side in the gale.

Very important, as supplementing my experiences at Rebecca Shoal, are observations made at Loggerhead Key by Dr. A. G. Mayer and other investigators. On July 11 they noted, under similar wind conditions to those at Rebecca Shoal, an unusual abundance of mosquitoes and preserved 22 specimens, which proved to be of the same species, *A. niger*. Dr. Mayer made the following record:

> "On the night of July 10, at about 8 p. m., there was a sudden squall with rain from the southeast. By the morning of July 11 the wind was south to south-southwest, blowing in a gentle south-southwest breeze all day. Mosquitoes were noticed at about noon of July 11, and became a pest by night, so that we were unable to lie on the sand, as is our custom, at the southern end of the island. Many of them were also found in the laboratory, and they were thick in the bushes near the buildings and over the ocean about 300 feet from shore on the west side of the island. During the night of July 11 and 12 the wind veered to southeast to east-southeast, and the mosquitoes practically disappeared."

Thus it appears that the migration of July 11, occurring simultaneously at Rebecca Shoal and Loggerhead Key, presented a front of at least 20 miles. The individuals which actually alighted upon the station were, of course, only a small percentage of those which crossed the open sea within the visual radius of 13 miles; only those which came very close could have been attracted by any slight odors emanating from the lighthouse. Although Loggerhead Key is three-fourths of a mile long and hence much larger than Rebecca Shoal station, it still represents a small area on the surface of the sea. From these data one hesitates to compute the possible numbers of mosquitoes that were carried out to sea by the wind blowing from the Cuban coast.

On July 18, I was transferred from Rebecca Shoal to the Tortugas, where observations were continued. The wind held in the east for the 10 days of my stay there, so that no opportunity arose to witness another mosquito migration. Attention was turned toward a determination of the numbers and species of the mosquitoes still present on the islands, and their possible breeding-places.

As stated by Dr. Mayer, the veering of the wind on the night of July 11–12 caused a marked decrease in the numbers of mosquitoes encountered in the vicinity of the laboratory; still it was possible 10 days later to attract considerable numbers of *Aëdes niger* by stationing one's self quietly in the shade near the bay cedar bushes. On July 22 mosquitoes in one of the laboratories were so numerous that 4 were taken in one sweep of the net. Again on July 23, in the half hour just preceding darkness, 11 specimens were caught.

Since there are no swamps on the small sandy keys, the only breeding-places for mosquitoes are such receptacles as may be maintained or left by man. Examination showed that no larvæ were present in the fresh-water storage reservoirs at the laboratory or at the Loggerhead light-station. It was thought likely that mosquitoes might be breeding in cans thrown upon a waste pile among the bay cedar bushes. On investigation many were found that contained rain-water but no larvæ. In short, not a single larva was found on Loggerhead Key during my visit. Nevertheless, one small batch had, earlier in the season, matured in a shallow pool on a cement floor beneath the refrigerator, and the possibility is not excluded that *Stegomyia calopus* may have been breeding there late in July; several specimens of this species were captured about the laboratory. But that *Aëdes niger* or *sollicitans* were breeding at that time is extremely doubtful, for both require brackish water for their development, and all the receptacles contained only fresh water. Not only in the vicinity of the laboratory was it easy to obtain specimens; they were distributed over the entire key. In the early morning, afternoon, and evening *A. niger* could be attracted from the grass and bay cedar bushes.

The conditions on another of the Tortugas Islands are interesting in this regard. East Key lies 6 miles east of Loggerhead and is the most isolated of the group as well as least frequently visited by man. Although it has a fairly luxuriant growth of bay cedars and xerophytic shrubs, there is not a drop of water on the key, and hence no possibility of mosquitoes breeding. On July 20 and 21 I found *Aëdes niger* so numerous at dusk and daybreak that as many as 4 females would be attempting to bite at one time. A walk through the grass at sunset was sure to arouse several of the insects. As to the origin of the mosquitoes found on East Key, two or three sources may have contributed. We have seen that on east winds a few of the *Aëdes niger* migrated to the Rebecca Shoal light-station. Since East Key is only 12 miles farther west it seems likely that a few may also reach this island from Marquesas, but, no doubt, the greater number came in the migrations of July 5 and 11. It is known that they are capable of living for some time in captivity, and so may well have survived on plant juices from July 11 to 21.

On July 23 an examination of the fresh-water reservoirs at Fort Jefferson was undertaken to determine the extent to which mosquitoes were breeding there. It was found that all except one of the tanks had been treated a short time before with kerosene, but this one contained large numbers of larvæ and pupæ. Several hundred were transferred in glass jars to Loggerhead Key, where some were preserved and the others allowed to mature.

Since *Stegomyia calopus* used to breed in the reservoirs at Fort Jefferson it was expected that at least a part of these would prove to be of that species. But all larvæ and adults reared from them proved to be *Culex pipiens* Linnæus. Not a single adult of this had been, or was later, taken either at Rebecca Shoal or at Tortugas. It is probable that the adults had been introduced by vessels visiting the fort and had deposited eggs in this reservoir. That the imagoes emerging from this source do not fly to other keys of the Tortugas 2 and 3 miles away is not surprising; *Culex pipiens* does not fly far from its breeding-place.

Unfortunately I did not succeed in determining the origin of the specimens of *Stegomyia calopus* which were taken on Loggerhead Key. The fact that the majority were males indicates that the breeding-place was not far away; indeed, it is known that this species seldom flies more than a few hundred yards.

THE MIGRATION OF THE HOUSE-FLY.

The migratory powers of *Musca domestica* were clearly demonstrated by the occurrence of specimens at Rebecca Shoal. On my arrival at the station, June 26, I found 6 house-flies; 2 more were taken on the following day. As previously explained, there was no possible breeding-place for *Musca domestica* on the lighthouse; all refuse is immediately thrown into the sea. So it was plain that these flies had either been introduced by boats or had flown across the sea. That boats are responsible for the presence of a few flies at the station was shown when the small auxiliary catboat brought one of the assistant keepers from Key West; 3 house-flies were found upon it as it was raised from the water to the platform. Mr. Roberts, the assistant, stated that the flies had annoyed him during the trip from the Marquesas Keys to Rebecca Shoal that day.

No more specimens were taken during the next 8 days, as will appear from the table on page 202. It is important to note that not even with the swarm of mosquitoes which arrived on July 5 were any house-flies noted. But on July 6, following the sudden change of the wind from north to east late on the preceding day, the lighthouse by contrast seemed fairly alive with *Musca domestica;* 25 were captured during the day—3 between 6 and 7^h30^m a. m.; 1 each at 9^h20^m, 9^h40^m, 10^h55^m, 11^h30^m, 11^h32^m; 10 between 11^h33^m and 1^h15^m; 2 at 2^h10^m p. m.; 1 at 3 p. m.; 2 at 4^h20^m; 1 at 5^h15^m, and the last at 5^h25^m.

I am at a loss to account for the flight of house-flies from the east, since no such numbers had come to the station on the east winds which prevailed from June 26 to July 2. Had they arrived along with the mosquitoes on July 5 the obvious assumption would have been that they had come from the coast of Florida, but the fact that none appeared until 12 hours after the wind had veered into the east seems rather to point to Marquesas or some island farther east along the reef as their source. That it would have been possible, as far as distance is concerned, for the flies to have been carried from Key West 46 miles to the east, will appear after a statement of the conditions under which the second notable migration of *Musca domestica* appeared at Rebecca Shoal, but the reason for their leaving an eastern point in such large numbers at this time is still hidden; possibly the sudden change in the direction of the wind took them unawares.

During the 7th, 8th, and 9th of July the wind continued to blow lightly from the east. On the 7th of July, 5 flies were taken, 2 of them at 5^h30^m a. m.; 1 at 6 and 2 at 12^h30^m p. m. July 8 brought 5 more, 1 at 9^h40^m a. m.; 2 at 12^h30^m; 1 at 2^h30^m, and 1 at 5^h20^m. A specimen was captured at 5^h10^m a. m. on July 9, another at 11^h30^m, and a third at 6 p. m.; none appeared on July 10 while the wind was shifting toward the south, but on the memorable 11th, along with 37 mosquitoes, came 18 house-flies from Cuba. It is interesting to note that, whereas the mosquitoes were captured at all hours of the day as they reached the station, these flies were all taken between 9^h50^m and 11^h42^m a. m., 1 hour and 52 minutes. A possible explanation suggests itself. It will be remembered that no *Musca domestica* accompanied the mosquitoes from the coast of Florida on the north wind of July 4 and 5, but the very shortest distance in that direction is 105 miles. Now, the distance from Rebecca Shoal to the coast of Cuba is 10 miles less or about 95 miles. These facts, taken with the sudden appearance and disappearance of the flies on July 11, suggests that 95 miles is about the limit to the distance over which they were able to sustain themselves with the wind blowing moderately. That the wind was not the only factor that caused the cessation of their appearance is plain from the fact that it neither changed its direction nor lessened its rate until several hours later.

On July 12 the number of *Musca domestica* fell to normal, 5 being taken. During the succeeding days of high east winds very few appeared at the lighthouse, 1 on July 13, 2 on the 14th, 1 on the 15th, and the last on the 17th.

At Tortugas house-flies are numerous at all times during the season when the Biological Laboratory is open. An attempt was made to determine whether or not they breed there. Since all sewage and refuse is at once disposed of in the sea, it was thought possible that *Musca domestica* might be breeding in dead land-crabs. In order to test this source 100 house-flies were inclosed within a glass jar containing 3 inches of sand, 2 large ghost crabs, *Ocypoda arenaria*, and a small dish of water. The jar was tightly closed with fine bolting-cloth to prevent the entrance of ants. Apparently no eggs were deposited, for no maggots developed.

When it was found that there was little likelihood of house-flies breeding at Tortugas, attention was turned to the question as to whether it would be reasonable to expect that all *Musca domestica* found there had either been introduced upon vessels—and many certainly are—or migrated with the aid of the winds. Now, to one who had formed his estimate of the numbers of house-flies present on Loggerhead Key by observations made in the laboratories, such a proposition might well seem unreasonable, but as a matter of fact the flies are practically limited to the laboratories and to the lighthouse buildings. I found hardly a half dozen specimens in the bushy and untenanted parts of the island. True to their name, the house-flies seek the buildings occupied by man. It is probable that the *Musca domestica* on Loggerhead Key during the latter part of July numbered not over 5,000. Calculating the number which may be expected to have been attracted to the island on the southerly wind from Cuba on July 11, by multiplying 25 taken at that time on Rebecca Shoal light-station by the relatively larger size of Loggerhead—say 2,000—and we have 50,000 flies, a number greatly in excess of those present. Hence it seems entirely within the range of possibility that *Musca domestica* does not breed on Loggerhead or the other Tortugas Keys, but that the few thousand specimens found there migrate from the mainland or from larger islands. Account should also be taken of the few flies which may reach Tortugas from the east; as high as 5 per day were often borne to Rebecca Shoal on easterly winds.

THE OCCURRENCE OF OTHER DIPTERA AT REBECCA SHOAL.

Besides mosquitoes and house-flies several species of Diptera visited Rebecca Shoal light-station during the period of observation. On June 29, with a moderate east wind blowing, a Tabanid of the sort commonly called greenheads settled upon the freshly painted railing of the platform, where it was captured; another was taken on July 7. Again the wind was blowing lightly from the east, indicating that these large flies were borne westward along the reef and were able to cross the 24 miles of open water between Marquesas and Rebecca Shoal station. *Tabanus* is not uncommon on Loggerhead Key.

Blow-flies of two species were also noted at the lighthouse and 5 were taken, 1 on June 27 and 2 on July 8, 1 on the 9th, and the last on the 10th. It will be seen from the table on page 202 that on all these days the winds were from the east.

The only other Dipteran which visited Rebecca Shoal was a small gnat taken just before dusk on July 2. Again the wind was in the east.

OTHER INSECTS NOTED AT REBECCA SHOAL STATION.

Other orders of insects were but meagerly represented at Rebecca Shoal. As noted above, a single damsel-fly was caught in paint applied to the building on June 21. A large dragon-fly was seen to approach the station on July 5; it alighted on the roof of the lantern at the very top of the structure, and when an attempt was made to capture it with a hand-net it escaped and disappeared to the southwest. Although a lookout was kept for it throughout the rest of the day nothing further was seen of the specimen, thus indicating that its long flight from the northward had not exhausted its strength. One specimen of the Neuropteran called green-eyed lace-wing, *Chrysopa*, came to the station on July 8.

One Lepidopteran made the passage from Cuba to Rebecca Shoal on the southerly winds which were responsible for the flights of mosquitoes and house-flies. On the morning of July 12, I had just reached the platform on the southwest side of the station when a large moth alighted upon one of the davits. Its capture proved it to be one of remarkable beauty and strength, *Syntomeida epilais*. Its form, similar to that of the Sphingidæ, suggested such speed and endurance as might well sustain it over a flight of 95 miles.

EVIDENCE THAT ODONATA AND LEPIDOPTERA MIGRATE TO TORTUGAS.

Mention has just been made of a dragon-fly which visited Rebecca Shoal station. At times these insects are numerous at Tortugas, both on Loggerhead and East Keys, where vegetation is abundant. In July 1914 within an hour I captured a score at East Key. On the island, whose extent is about 6 acres, there were at that time at least

100, but during the two days spent there the past season only 2 were seen, 1 of them being captured. At Loggerhead on July 19 a single individual was the reward of a day's lookout, and only 3 were seen there until the morning of July 30, when, as we were closing the laboratories preparatory to leaving the island, the air over the bay-cedar bushes near the buildings was alive with dragon-flies. I counted a score in sight at one time.

Now, since there is no possible breeding-place for Odonata upon any of the Tortugas Islands, it is plain that the great variation in numbers must be due to their migratory habits; for it is not likely that boats or other agencies are responsible for their introduction. As an example of the tenacity with which dragon-flies follow vessels upon which they may chance to be carried from shore, the case may be cited of a large specimen observed flying about the superstructure of the steamship *Concho* just before it left the dock at Key West. This insect, or one of its species, was noted on the following day while the boat was passing through the Straits of Florida. It was still on board when, toward the end of the voyage, the vessel was within 100 miles of New York.

Occasionally single specimens of Lepidoptera appear at Tortugas as if brought involuntarily from distant haunts. On July 28 a Sphingid, *Sesia tantalus* Linnæus, was captured while feeding on lilies near the laboratory. While certain species apparently breed on Loggerhead and East Keys, others are so rare as to indicate that they are immigrants. Dr. Mayer is authority for the statement that one of the larger Noctuidæ often comes to Tortugas on southerly winds from Cuba.

DO SARCOPHAGIDÆ BREED AT TORTUGAS?

Conspicuous by their numbers on the open sandy shores and among the vegetation closely bordering them at Tortugas are two species of Sarcophagidæ or flesh-flies. Mr. R. R. Parker has identified the larger as *Sarothromyia femoralis* Schiner, and the smaller as *Sarcophagula occidua* Fabricius. Like most of the insects inhabiting these white beaches where the light is intense, both species are much lighter in color than ordinary members of the family. Their flight is also peculiar in so far as I am familiar with the group. As one walks along the beach these flies dart into the air only to alight a few feet in advance, repeating these short flights almost as a series of hops if disturbed by the progress of the intruder. I was unable to cause an extended flight. The possibility was suggested that their powers of flight had been modified after dwelling for some time on these isolated keys. If such were true then certainly they must be breeding there, for their numbers are considerable.

An experiment was performed in order to ascertain whether or not these Sarcophagids would breed in dead land-crabs. On July 19 a glass jar containing 3 inches of sand and a freshly killed ghost crab, *Ocypoda* *arenaria*, whose carapace measured 1½ inches, was set under bay cedars near the beach and several specimens representing both species of flies were inclosed by means of a cover of fine bolting cloth. Within the next 2 days all the adults died and none was seen inside the jar until the morning of July 29, when three imagoes had emerged. On examination 101 pupæ were found near the bottom of the sand; the majority were of the larger species. It is thus evident that, if 104 maggots of a fly the size of the house-fly can develop to maturity within a single small crab, it is not necessary to look to migrations to keep up the stock of adults at Tortugas. To be sure, the large number of ants there may be relied upon to keep the flies within bounds; very few dead crabs are left long above ground. But years ago a former keeper of Loggerhead Light, Mr. George Billbury, called Dr. Mayer's attention to the fact that certain flies were to be noted about the entrances of crab burrows in the sand. As he surmised at that time, it is very possible that these flies may occasionally breed in crabs which, for some reason, die in their burrows.

SUMMARY.

1. Large numbers of mosquitoes and house-flies are carried by northerly and southerly winds to Rebecca Shoal light-station and the Tortugas Islands from Florida and Cuba.
2. Easterly winds bring a few of these, as well as smaller numbers of blow-flies, horse-flies, and gnats from islands east on the Florida Reef.
3. Occasionally Odonata, Neuroptera, and Lepidoptera are carried by the winds to these parts of the reef.
4. Sarcophagidæ breed in land crabs at Tortugas.

BIBLIOGRAPHY.

Coquillet, D. W. 1905. A classification of the mosquitoes of North and Middle America. Technical Series No. 11, Bureau Entomol., U. S. Dept. of Agric.

Giles, George M. 1900. A handbook of the gnats or mosquitoes. xi+374 pages.

Howard, L. O. 1901. Mosquitoes. xviii+241 pages.

———., Harrison G. Dyar, and Frederick Knab. The mosquitoes of North and Central America and the West Indies. In 4 volumes. Octavo. Carnegie Inst. Wash. Pub. No. 159.

Vol. 1. A general consideration of mosquitoes, their habits and their relations to the human species. vii+520 pages, 14 plates, 6 text figures. 1912.
Vol. 2. x pages, 150 plates. 1912.
Vol. 3. Systematic description, Part I. Pages vi+523. 1915.
Vol. 4. Systematic description, Part II. Pages 525 to 1064. 1917.

Mitchell, Evelyn G. 1907. Mosquito life.

Smith, J. B. 1904. Report of the New Jersey State Agricultural Experiment Station upon the mosquitoes occurring within the State, etc. 482 pages.

Theobald, F. V. 1901. A monograph of the Culicidæ of the world. Vols. 1 and 2, British Museum.

XI.

ON CHANGES IN THE SEA AND THEIR RELATION TO ORGANISMS.

BY J. F. McCLENDON,

Associate Professor of Physiology, University of Minnesota Medical School.

Eight figures.

CONTENTS.

PART I.

PAGE.
Introduction 215
Methods 218
Currents 221
Records of all stations and of a tank of sea-water 222
Discussion 227

PART II.

Relation of oxygen tension and activity of the nervous system to metabolism in Cassiopea 235
On the chemical precipitation of $CaCO_3$ in sea-water 252
Bibliography 258

ON CHANGES IN THE SEA AND THEIR RELATION TO ORGANISMS.

By J. F. McClendon.

PART I.

INTRODUCTION.

The sea and air form the circulating media for the living organisms of the world, and although the air circulates so rapidly that no correlation between local faunas and floras and local composition of the air has been found, the local composition of the sea is distinctly affected by living organisms. The local changes in the composition of the sea are the subject of the present paper. These changes are due chiefly to organisms, but partly to meteorological causes. The circulation of the air certainly affects the sea, but the circulation is so rapid that my attempts at correlating meteorological observations (made for this purpose) with changes in the sea have not been encouraging.

The water evaporated is returned with addition of fixed nitrogen from electric discharges or falls on the land and is returned with various salts, chiefly $CaCO_3$, and with fixed nitrogen and other products of organisms. Various seaweeds absorb CO_2, thus leaving an excess of $CaCO_3$, which has a very low solubility and is constantly being precipitated in certain warm seas and is precipitated within the bodies of organisms in the surface waters of all seas. In working out the relation of H-ion concentration (pH) to the solubility of $CaCO_3$ in sea-water, I found that all sea-water is supersaturated with $CaCO_3$, and will lose some of it if shaken with calcite or aragonite crystals.

The study of the local changes in the sea is complicated by the presence of currents. The surface currents are due to winds, but, owing to the inertia of the water, they do not change as rapidly as the wind and hence are indicators of the prevailing winds (fig. 1), except where they are deflected by bodies of land or rotation of the earth. The largest currents may be constant (fig. 2), but the smaller currents show seasonal variations. Very few constant or seasonal vertical currents have been mapped, but vertical currents must be universal. McEwen has collected an enormous mass of evidence to show the presence of vertical currents off the California coast. Vertical currents occur also off the coast of Norway and in the eastern Mediterranean in winter. I have found local and diurnal changes in the O_2 and CO_2 content of the sur-

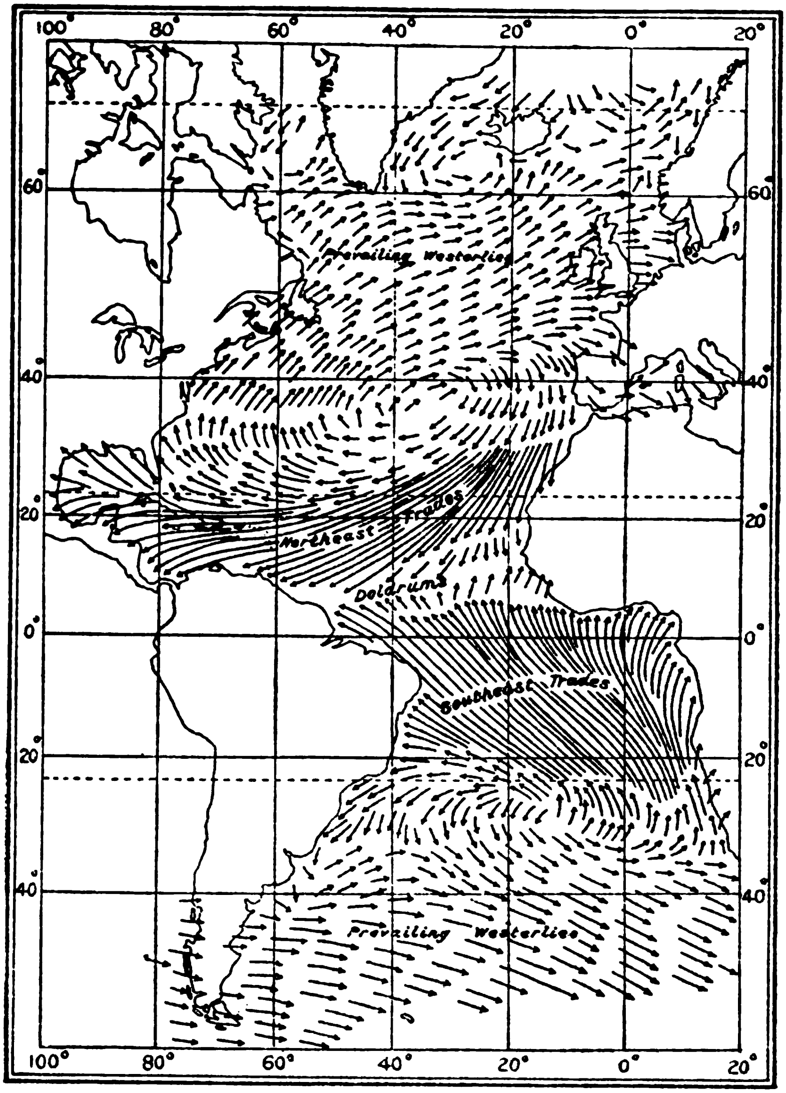


FIG. 1.—Winds of the Atlantic Ocean. The long arrows show directions of steady winds, the short arrows the prevailing directions of variable winds.

face correlated with the presence of plants attached to the bottom (off the Florida Keys), indicating a complete inversion of the water or mixing of top and bottom water several times a day; the mixing was not rapid enough, however, to equalize the temperature to the depth of 50 meters on a calm sunny day. Palitzsch (1912 *b*) found the average

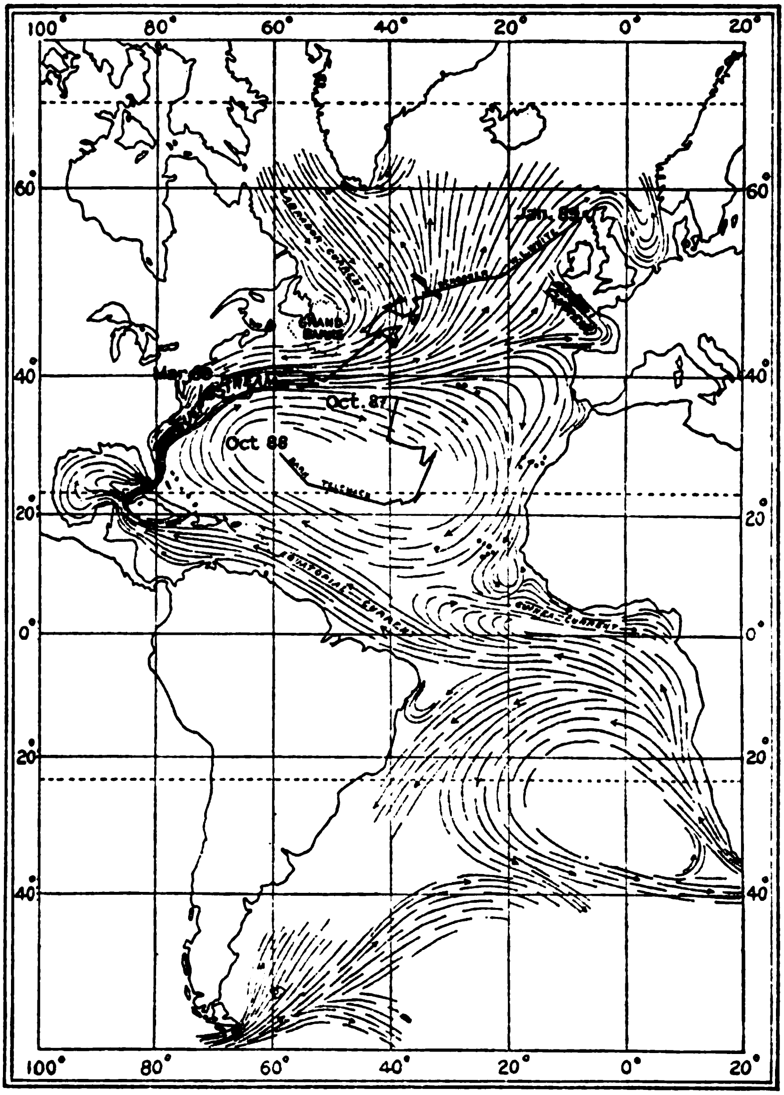


Fig. 2.—Currents of the Atlantic Ocean. The arrows show direction. The heavy lines are the courses of two derelicts, drifted by currents and winds.

O_2 in surface water of the North Atlantic to be 5.9 c.c. per liter during the day and 5.34 at night.

METHODS.

The opportunities offered permitted of continuous observations at two stations, stations A and B, figure 4 (east and west of Loggerhead Key, Dry Tortugas), more or less systematized observations around Tortugas (fig. 4), and a series of observations on board between Tortugas and New York City (fig. 3).

The number of grams of chlorine per kilogram of sea-water (abbreviated Cl) was determined by titration with silver-nitrate solution, using potassium chromate as indicator and standardizing the whole method with sea-water standardized by the International Commission. The number of cubic centimeters of O_2 per liter was determined by the Winkler method. The self-closing water-bottle was not adequate for this purpose, but some observations were made on water drawn up from about 60 meters through glass and rubber tubing attached to the wire of a Lord Kelvin sounding-machine, and run continuously through the analysis bottle before reaching the pump. Twice the volume of the tube was run through the sample bottle with the least possible suction before the analysis was made. The alkaline reserve was recorded as the number of cubic centimeters of 0.01 N HCl used in titrating 100 c.c. of sea-water while boiling in a 500 c.c. Erlenmeyer "nonsol" flask, using di-brom-o-cresol-sulfonphthalein as indicator, until all trace of purple color had disappeared. The end-point is not sharp, but is sharper with this indicator than with any other yet found. The water must not dry on sides of flask. The pH was determined colorimetrically (McClendon, 1917), correction being made for Cl. The total CO_2 per liter was determined from the alkaline reserve and pH by means of the conversion table.

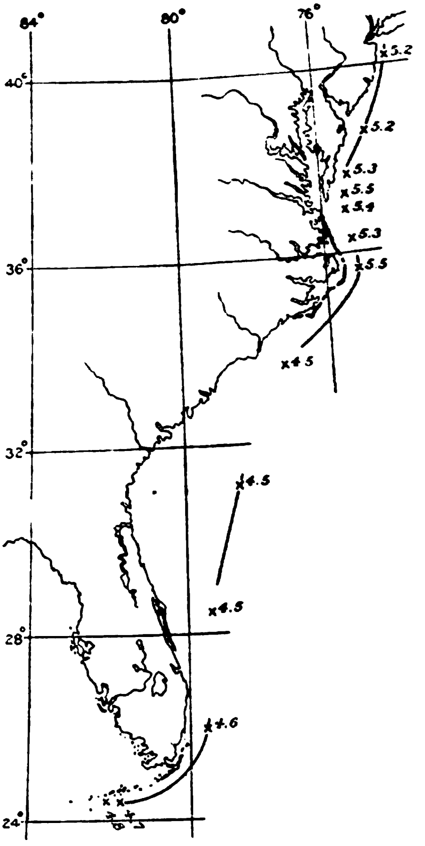


FIG. 3.—Oxygen in surface water taken on voyage from Key West to New York. The track of the steamer at night is marked by heavy black lines. The numbers denote the cubic centimeters of O_2 per liter at the stations marked *x*.

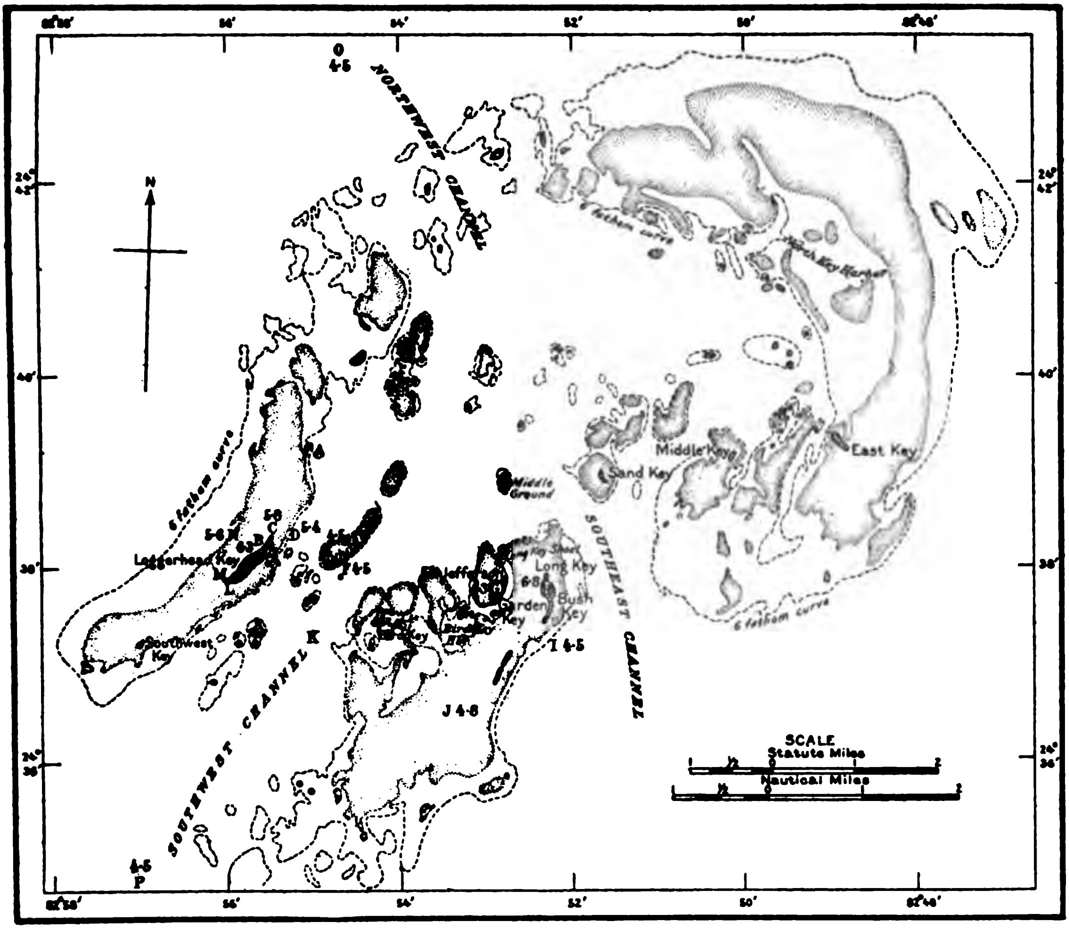


FIG. 4.—Positions of stations A to N at Tortugas, Florida. The numbers opposite the letters A to N denote the cubic centimeters O_2 per liter in the afternoon.

Table 1 gives the total CO_2 for alkaline reserve = 25.

TABLE 1.

pH.	Total CO_2.	pH.	Total CO_2.	pH.	Total CO_2.
8.17	45.0	8.26	43.65	8.35	42.29
8.18	44.9	8.27	43.5	8.36	42.14
8.19	44.7	8.28	43.35	8.37	41.99
8.20	44.55	8.29	43.19	8.38	41.80
8.21	44.4	8.30	43.04	8.39	41.75
8.22	44.3	8.31	42.89	8.40	41.60
8.23	44.15	8.32	42.74	8.41	41.45
8.24	44.00	8.33	42.59	8.42	41.30
8.25	43.8	8.34	42.44	8.43	41.15

The CO_2 of the air was determined colorimetrically (McClendon, 1917), 30 observations showing it to vary from 2.8 to 3.5 parts per

10,000. A new aneroid barometer was used and the mean value for 2 months was taken as 760. A thermometer graduated down to tenths of a degree was standardized by the United States Bureau of Standards and the other thermometers compared with it. A recording thermometer for the air was compared with the mercury thermometer, but the records were not found to be of much value in relation to the sea. Tortugas is about on the heat equator during July. There was a diurnal variation of the temperature of the air from 25° at dawn to 30° at 1 p. m., with a very slight upward drift during July and with a few variations due to storms. All time records are in local apparent time. The weights used were standardized by the United States Bureau of Standards and the glass apparatus and solutions standardized by weighing.

All water samples that could not be examined immediately were preserved in large flasks of resistance glass entirely full of the sample and were examined as soon as possible. Most of the samples taken at night were titrated by artificial light. The pH was in such cases determined with the aid of a "daylite" lamp, but the results were not very satisfactory.

Samples (for pH) taken a short time before sunrise were kept in nonsol flasks for the pH to be determined after sunrise.

The CO_2 tension at 30° was found from the pH, using table 2. The correction for other temperatures was made possible by the fact that a change of 1° temperature corresponds to 0.01 pH. The unit of CO_2 tension is one ten-thousandth of a normal atmosphere (760 mm. Hg) of CO_2.

TABLE 2.

pH.	CO_2 tension.	pH.	CO_2 tension.	pH.	CO_2 tension.
8.20	3.4	8.26	2.8	8.32	2.3
8.21	3.3	8.27	2.7	8.34	2.2
8.22	3.2	8.28	2.7	8.36	2.1
8.23	3.1	8.29	2.6	8.38	2.0
8.24	3.0	8.30	2.5	8.40	1.9
8.25	2.9	8.31	2.4		

The sea-water was usually supersaturated or undersaturated with oxygen. The number of cubic centimeters of O_2 per liter at saturation at a given temperature was read from figure 5 and the excess or deficiency of saturation of the water was recorded. Figure 5 compares favorably with the results of Fox, who used a different method of analysis. Jacobsen's formula for cubic centimeters O_2 per liter at saturation is $10.062-0.2822\ t-0.006144\ t^2-0.000061\ t^3-0.1073\ Cl+0.003586\ tCl-0.000055\ t^2Cl$. t = temperature and Cl = grams chlorine per kilogram of sea-water.

The determinations for constructing this curve were made as follows: A wash-bottle of about 500 c.c. capacity was supplied with about 400 c.c. of sea-water and immersed in a thermostat. Moistened outdoor air was drawn through the wash-bottle by means of a suction-pump until equilibrium was established. The air passed through a very small wash-bottle of sea-water and a copper coil (both immersed in the thermostat) before reaching the 500 c.c. wash-bottle. The analyses were made by the Winkler method, using the same standards as with the other determinations, a correction being applied for the average gas-pressure in the wash-bottle. No correction was made for the vapor tension of water, since it was the same as at the sea surface everywhere.

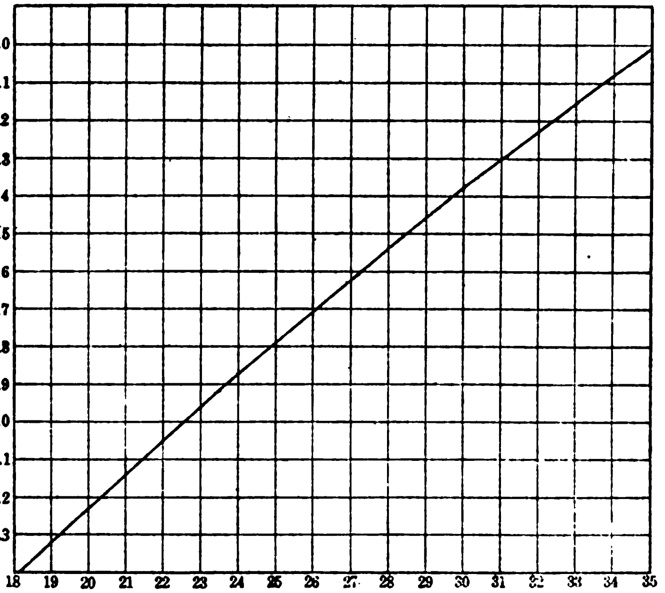


Fig. 5.—Curve of equilibrium of Tortugas sea-water with air at 760 mm. barometric pressure; the degrees temperature (18 to 35) are marked on the abscissa and the number of cubic centimeters of oxygen per liter of sea-water (5.3 to 4.0) on the ordinate.

CURRENTS.

The surface currents to be considered are:

(1) The Gulf Stream, which parallels the Florida Keys and then flows toward Cape Hatteras (but only those observations between the latitudes of Miami, Florida, and Wilmington, North Carolina, were in the axis of the stream).

(2) The countercurrent in shallow water, which flows from Cape Hatteras, along the shore, until it reaches the Florida Keys, when it flows in the Hawk Channel (between the keys and the outer reef) and passes through the Tortugas and out into the Gulf of Mexico.

(3) The tidal currents. In the region of the Florida Keys the ebb is south and the flow north.

At Tortugas, the ebb flows through the northwest channel and continues out through the southeast and southwest channels, with a maximum speed of about 2 knots, as measured by Vaughan (1910, 1914), so that it does not go more than 12 miles before returning, and hence does not reach the Gulf Stream. But it seems probable that a considerable admixture of Gulf Stream water finally reaches Tortugas.

The time the tide turned, starting south (high water), at Tortugas is given in table 3 for July 1917.

TABLE 3.

Day.	A. M.	P. M.	Day.	A. M.	P. M.	Day.	A. M.	P. M.
1	$5^{h}12^{m}$	$7^{h}07^{m}$	12	$2^{h}41^{m}$	$3^{h}45^{m}$	22	$10^{h}35^{m}$	$11^{h}19^{m}$
2	6 02	7 56	13	3 42	4 59	23	11 12	11 49
3	6 53	8 35	14	4 41	6 04	24	11 52	.....
4	7 42	9 15	15	5 38	6 39	25	12 11	12 41
5	8 32	9 54	16	6 29	7 46	26	12 38	1 53
6	9 21	10 34	17	7 18	8 28	27	1 17	3 25
7	10 14	11 16	18	7 57	9 06	28	2 16	4 47
8	11 08	.....	19	8 43	9 42	29	3 28	5 51
9	12 01	12 07	20	9 16	10 16	30	4 41	6 43
10	12 50	1 14	21	9 59	10 49	31	5 45	7 26
11	1 43	2 28						

RECORDS OF ALL STATIONS AND OF A TANK OF SEA-WATER.

Table 4 gives the determinations of the surface water at station A (fig. 4) on the eastern side of Loggerhead Key for the month of July 1917, and table 5 gives similar data of a tank of sea-water 4 by 6 by 4 feet for July 23–24. The unit of CO_2 tension is one ten-thousandth of a normal atmosphere of CO_2 (760 mm. Hg).

TABLE 4, STATION A.

Date.	Time of day.	Temp.	pH.	Total CO_2.	CO_2 tension.	O_2 per liter.	O_2 saturation, excess or deficiency.
July 2..	$5^{h}00^{m}$p. m.	28.0	8.26	43.65	2.6	5.35	+0.81
3..	8 30 a. m.	26.7	8.18	44.9	3.3	4.5	−0.15
3..	4 30 p. m.	28.0	8.26	43.65	2.6	5.35	+0.81
4..	6 30 a. m.	27.6	8.20	44.65	3.2	4.52	−0.06
4..	5 30 p. m.	28.5	8.26	43.65	2.6	5.7	+1.20
5..	6 30 a. m.	27.8	8.22	44.30	3.0	4.52	−0.04
5..	6 10 p. m.	28.6	8.25	43.80	2.8	4.00	+1.00
6..	6 30 a. m.	27.9	8.20	44.55	3.2	4.55	0.00
6..	5 30 p. m.	28.8	8.25	43.80	2.8	5.35	+0.87
7..	6 30 a. m.	27.7	8.19	44.70	3.3	4.44	−0.13
7..	5 30 p. m.	28.8	8.25	43.80	2.8	5.35	+0.87
8..	6 30 a. m.	27.5	8.20	44.55	3.2	4.3	−0.25
8..	5 30 p. m.	29.0	8.27	43.5	2.6	6.1	+1.64

TABLE 4, STATION A—Continued.

Date.	Time of day.	Temp.	pH.	Total CO_2.	CO_2 tension.	O_2 per liter.	O_2 saturation, excess or deficiency.
July 9..	6^{h}30^m a. m.	28.1	8.20	44.55	3.2	4.26	−0.27
9..	6 00 p. m.	29.1	8.28	43.34	2.6	5.97	+1.52
10..	5 30 a. m.	28.2	8.20	44.55	3.2	3.96	−0.57*a*
10..	6 30 a. m.	28.4	....	.....	...	4.21	−0.30*a*
10..	8 30 a. m.	28.8	....	.....	...	4.47	−0.01*a*
10..	10 00 a. m.	29.2	....	.....	...	4.90	+0.45*a*
10..	11 00 a. m.	29.6	8.25	43.8	2.9	5.2	+0.79*a*
10..	12 00 noon.	29.5	8.25	43.8	2.9	5.2	+0.78
10..	1^{h}30^m p. m.	29.7	8.26	43.65	2.8	5.2	+0.80*a*
10..	3 15 p. m.	30.0	8.26	43.65	2.8	5.6	+1.22*a*
10..	4 15 p. m.	30.2	8.27	43.50	2.7	5.8	+1.44*a*
10..	5 15 p. m.	29.5	8.27	43.50	2.7	5.5	+1.08*a*
10..	6 15 p. m.	29.0	8.26	43.65	2.7	5.43	+p.97*a*
10..	7 00 p. m.	28.8	8.26	43.65	2.7	5.13	+0.65*a*
10..	9 00 p. m.	28.3	....	.....	...	4.52	0.00*b*
11..	2 50 a. m.	28.1	....	.....	...	4.25	−0.28
11..	5 50 a. m.	28.0	8.22	44.3	3.0	4.13	−0.41
11..	6 30 a. m.	28.2	....	.....	...	4.20	−0.32
11..	2 10 p. m.	30.0	8.26	43.65	2.8	5.2	+0.82
11..	4 30 p. m.	29.5	8.26	43.65	2.8	5.3	+0.88
12..	6 45 a. m.	28.6	8.23	44.15	3.0	4.35	−0.14
12..	12 15 p. m.	30.5	8.25	43.8	2.9	5.3	+0.96
12..	4 00 p. m.	30.5	8.27	43.5	2.7	5.3	+0.96
12..	5 00 p. m.	30.2	8.27	43.5	2.7	4.97	+0.61
13..	6 30 a. m.	28.1	8.18	44.9	3.4	4.12	−0.88*c*
13..	12 30 p. m.	29.5	8.25	43.8	2.9	4.85	+0.43*c*
13..	5 20 p. m.	29.7	8.26	43.65	2.8	4.85	+0.45*c*
14..	5 45 a. m.	28.3	8.20	44.55	3.2	4.5	−0.02*c*
14..	4 00 p. m.	29.5	8.26	43.65	2.8	4.73	+0.31*c*
15..	6 00 a. m.	28.2	8.21	44.4	3.1	4.14	−0.39*c*
15..	4 20 p. m.	29.8	8.28	43.34	2.6	4.5	+0.10*c*
16..	6 45 a. m.	28.0	8.21	44.4	3.1	4.4	−0.14*c*
16..	4 15 p. m.	29.8	8.27	43.5	2.7	4.84	+0.44*c*
17..	6 25 a. m.	28.0	8.20	44.55	3.2	4.33	−0.23
17..	4 55 p. m.	30.1	8.27	43.5	2.7	5.00	+0.63
18..	6 00 a. m.	28.0	8.20	44.55	3.2	4.28	−0.26
18..	4 00 p. m.	30.0	8.25	43.8	2.9	4.85	+0.47
19..	5 20 a. m.	28.1	8.21	44.40	3.1	4.30	−0.23
19..	4 05 p. m.	29.4	8.24	44.00	3.0	4.62	+0.19
19..	4 30 p. m.	29.3	8.24	44.00	3.0	4.84	+0.44
19..	5 00 p. m.	29.3	....	.....	...	4.93	+0.49
19..	5 40 p. m.	29.7	....	.....	...	4.87	+0.43
19..	6 05 p. m.	29.9	....	.....	...	4.97	+0.58
19..	6 30 p. m.	29.7	....	.....	...	4.84	+0.44
19..	6 45 p. m.	29.4	....	.....	...	4.89	+0.42
19..	9 35 p. m.	28.6	8.22	44.30	3.1	4.45	−0.04
20..	6 15 a. m.	28.0	8.21	44.4	3.1	4.35	−0.19
20..	6 20 a. m.	27.9	....	.....	...	4.40	−0.15
20..	6 55 a. m.	28.0	....	.....	...	4.37	−0.17
20..	8 25 a. m.	28.4	8.22	44.30	3.0	4.58	+0.07
20..	9 25 a. m.	28.6	....	.....	...	4.67	+0.18
20..	10 30 a. m.	28.9	....	.....	...	4.58	+0.11
20..	11 30 a. m.	29.0	8.22	44.30	3.1	4.73	+0.27
20..	12 15 p. m.	29.2	....	.....	...	4.70	+0.25
20..	2 00 p. m.	28.9	8.22	44.30	3.1	4.70	+0.23
20..	3 30 p. m.	28.7	8.22	44.30	3.1	4.70	+0.22
20..	6 10 p. m.	29.8	8.24	44.00	2.9	4.70	+0.22
21..	5 45 a. m.	28.0	....	.....	...	3.94	−0.60*d*

a Unusually calm. *b* Sudden squall. *c* Rough weather. *d* Squall.

TABLE 4, STATION A—Continued.

Date.	Time of day.	Temp.	pH.	Total CO_2.	CO_2 tension.	O_2 per liter.	O_2 saturation, excess or deficiency.
July 21..	6^h30^ma. m.	27.9	8.20	44.55	3.0	4.12	−0.43
21..	9 30 a. m.	28.2	8.20	44.55	3.2	4.50	−0.02
21..	11 40 a. m.	28.7	....	.....	...	4.63	+0.15
21..	2 36 p. m.	29.4	8.22	44.30	3.2	4.63	+0.20
21..	4 30 p. m.	29.1	8.22	44.30	3.1	4.61	+0.16
21..	6 20 p. m.	29.2	8.23	44.15	3.0	4.77	+0.33
22..	5 20 a. m.	28.2	8.22	44.30	3.0	4.13	−0.39
22..	9 15 a. m.	28.6	....	.....	...	4.55	+0.06
22..	2 30 p. m.	29.6	8.25	43.80	2.9	4.76	+0.35
22..	4 30 p. m.	29.2	8.25	43.80	3.0	4.68	+0.23
22..	6 15 p. m.	29.0	8.23	44.15	3.0	4.60	+0.14
22..	9 30 p. m.	28.7	....	.....	...	4.32	−0.16
23..	5 40 a. m.	28.2	8.20	44.55	3.2	4.07	−0.45
23..	6 45 a. m.	28.2	....	.....	...	4.15	−0.37
23..	8 45 a. m.	28.7	8.21	44.40	3.2	4.62	+0.13
23..	10 45 a. m.	29.3	8.24	44.00	2.9	4.76	+0.32
23..	12 10 p. m.	29.9	....	.....	...	4.77	+0.38
23..	2 10 p. m.	29.8	8.25	43.80	2.9	4.87	+0.47
23..	3 55 p. m.	29.5	8.25	43.80	2.9	4.81	+0.39
23..	5 45 p. m.	29.2	8.23	44.15	3.0	4.72	+0.27
23..	7 10 p. m.	28.9	....	.....	...	4.55	+0.08
23..	9 10 p. m.	28.7	8.21	44.4	3.2	4.30	−0.17
24..	2 30 a. m.	28.5	8.21	44.40	3.2	4.15	−0.35
24..	4 50 a. m.	28.3	8.20	44.55	3.2	4.10	−0.42
24..	5 40 a. m.	28.3	8.20	44.55	3.2	4.15	−0.37
24..	7 35 a. m.	28.5	8.22	44.30	3.1	4.31	−0.19
24..	12 40 p. m.	29.8	8.25	43.80	2.9	4.88	+0.48
24..	3 30 p. m.	30.4	8.24	44.00	3.0	4.77	+0.42
24..	4 45 p. m.	29.9	8.23	44.15	3.1	4.76	+0.37
25..	5 30 a. m.	28.6	8.21	44.4	3.3	4.04	−0.45
25..	10 00 a. m.	29.0	8.22	44.3	3.3	4.60	+0.14
25..	11 30 a. m.	29.6	8.26	43.65	2.8	4.72	+0.31
25..	12 15 p. m.	29.8	8.26	43.65	2.8	4.72	+0.32
25..	2 35 p. m.	30.6	8.26	43.65	2.9	4.87	+0.54
25..	4 20 p. m.	30.3	8.26	43.65	2.8	5.29	+0.93
26..	6 15 a. m.	28.5	8.22	44.30	3.3	4.22	−0.28
26..	12 40 p. m.	29.4	8.24	44.00	3.1	4.86	+0.43
26..	4 15 p. m.	29.8	8.20	43.80	2.9	4.82	+0.42
27..	6 40 a. m.	28.3	8.20	44.55	3.5	3.82	−0.70
27..	1 40 p. m.	30.5	8.25	43.80	3.0	5.26	+0.92
27..	3 50 p. m.	30.7	8.25	43.80	3.0	5.06	+0.73
28..	6 15 a. m.	28.5	8.20	44.55	3.5	4.10	−0.40
28..	1 30 p. m.	30.5	8.26	43.65	2.8	5.10	+0.76
28..	2 50 p. m.	30.5	8.26	43.65	2.8	5.20	+0.86
28..	5 45 p. m.	30.2	8.27	43.50	2.7	4.78	+0.42
29..	6 30 a. m.	28.7	8.22	44.30	3.3	4.18	−0.30
30..	5 30 a. m.	28.8	....	.....	...	4.42	−0.06

TABLE 5, TANK.

Date.	Time of day.	CO_2 tension of air.	Temp.	pH.	Total CO_2.	CO_2 tension.	O_2 per liter.	O_2 saturation, excess or deficiency.
July 23....	5^h40^ma. m.	2.8	28.0	8.18	44.9	3.4	3.11	−1.14
23....	6 45 a. m.	...	28.5	....	....	...	3.43	−1.07
23....	8 45 a. m.	2.8	29.2	8.19	44.7	3.4	3.71	−0.73
23....	10 45 a. m.	2.8	30.0	8.21	44.4	3.3	4.08	−0.30
23....	12 10 p. m.	...	32.5	8.21	44.4	3.5	4.50	+0.31
23....	2 10 p. m.	2.8	34.5	8.24	44.0	3.4	5.01	+0.97
23....	3 55 p. m.	2.8	35.2	8.26	43.7	3.3	5.25	+1.25
23....	5 45 p. m.	2.9	35.1	8.25	43.8	3.4	5.30	+1.30
23....	7 10 p. m.	...	33.8	....	....	....	5.07	+0.98
23....	9 10 p. m.	...	32.5	8.22	44.3	3.4	4.76	−0.57
24....	2 30 a. m.	...	29.7	8.21	44.4	3.3	4.36	−0.08
24....	4 50 a. m.	...	28.6	8.20	44.6	3.3	3.30	−0.19
24....	5 40 a. m.	...	28.5	8.18	44.9	3.5	3.26	−0.24
24....	7 35 a. m.	...	28.7	8.20	44.6	3.3	3.48	−1.00

Table 6 shows the determinations at stations B to P as located in figure 4.

TABLE 6.

Station.	Day of month.	Hour.	Temp.	pH.	Total CO_2.	CO_2 tension.	Oxygen per liter.	O_2 saturation, excess or deficiency.
B	July 25....	10^h00^ma. m.	28.9	8.22	44.30	3.3	4.26	−0.21
B	25....	11 30 a. m.	29.4	8.26	43.65	2.7	5.37	+0.94
B	25....	12 15 p. m.	29.6	8.27	43.50	2.7	5.51	+1.1
B	25....	2 35 p. m.	30.2	8.31	42.89	2.5	6.46	+2.10
B	25....	4 20 p. m.	30.0	8.33	42.59	2.2	6.29	+1.91
B	26....	6 15 a. m.	28.5	8.20	44.55	3.3	3.58	−0.92
B	26....	12 40 p. m.	29.4	8.25	43.80	2.9	4.91	+0.48
B	26....	4 15 p. m.	29.9	8.34	42.44	2.2	6.10	+1.71
B	27....	6 40 a. m.	28.2	8.18	44.90	3.4	3.33	−0.19
B	27....	1 40 p. m.	30.0	8.32	42.74	2.3	6.55	+1.73
B	27....	3 50 p. m.	30.6	8.35	42.29	2.2	6.81	+1.47
B	28....	6 15 a. m.	28.5	8.19	44.70	3.4	3.89	−0.61
B	28....	1 30 p. m.	29.9	8.32	42.74	2.3	6.18	+1.79
B	28....	2 50 p. m.	30.2	8.32	42.74	2.3	6.16	+1.80
B	28....	3 30 p. m.	30.5	8.32	42.74	2.4	6.28	+1.94
B	28....	5 45 p. m.	30.8	8.31	42.89	2.5	5.55	+1.23
B	29....	6 30 a. m.	28.6	8.22	44.30	3.1	3.87	−0.62
B	30....	5 30 a. m.	28.6	....	.....	...	3.59	−0.90
C	28....	3 30 p. m.	30.5	8.32	42.74	2.4	6.28	+1.94
D	28....	3 30 p. m.	30.2	8.27	43 50	2.7	5.43	+1.07
E	28....	3 10 p. m.	29.3	8.24	44.00	2.9	4.53	+0.09
F	28....	10 35 a. m.	....	8.23	44.15	...	4.58	......
F	28....	3 40 p. m.	29.3	8.24	44.00	2.9	4.46	+0.02
F	29....	10 35 a. m.	....	8.22	44.30	...	4.37	......
G	28....	9 00 a. m.	....	8.20	44.55	...	4.05	......
G	28....	4 00 p. m.	31.3	8.32	42.74	2.4	6.28	+2.00
H	28....	4 20 p. m.	34.6	8.43	41.15	2.0	6.81	+2.77
I	28....	4 40 p. m.	29.4	8.23	44.15	3.0	4.51	+0.08
J	28....	4 55 p. m.	29.5	8.24	44.00	3.0	4.84	+0.42
K	28....	5 10 p. m.	29.3	8.24	44.00	2.9	4.63	+0.19
L	28....	5 22 p. m.	29.5	8.25	43.80	2.9	4.72	+0.30
M	28....	5 30 p. m.	29.9	8.25	43.80	2.9	5.07	+0.68
N	25....	4 10 p. m.	29.7	8.30	43.04	2.5	5.45	+1.05
O	18....	12 30 p. m.	28.5	8.22	44.30	3.1	4.5	0.00
[1]O	18....	12 30 p. m.	26.0	8.18	44.90	3.2	....	......
P	20....	4 00 p. m.	30.0	8.24	44.00	3.0	4.54	+0.16
[2]P	20....	4 00 p. m.	....	8.24	44.00	...	4.51	......

[1] Water collected at 50 meters depth. [2] Water collected at 65 meters depth.

In table 7 are given the determinations made on board, during the return from Tortugas to New York, together with the latitude and longitude of the stations.

TABLE 7.

Date.	Lat.	Long.	Time of day.	Temp.	pH.	Oxygen per liter.	Cl per kilo.	Alkaline reserve.	CO_2 tension.
July 30..	24°36′N.	82°48′W.	9^h00^m a. m.	29.0	8.21	4.48	....	....	...
	24 36 N.	82 41 W.	10 00 a. m.	29.6	8.21	4.59	....	....	...
	24 35 N.	82 35 W.	11 00 a. m.	28.2	8.21	4.80	....	....	...
	24 35 N.	82 34 W.	11 10 a. m.	27.7	8.21	4.80	....	....	...
	24 34 N.	82 28 W.	12 noon.	29.5	8.21	4.77	....	....	...
	24 33 N.	82 21 W.	1^h00^m p. m.	29.3	8.21	4.75	....	....	...
	24 33 N.	82 07 W.	4 00 p. m.	35.4	8.46	6.00	....	....	...
	24 33 N.	82 08 W.	4 10 p. m.	34.5	8.46	6.00[1]	....	....	...
	24 30 N.	82 00 W.	5 00 p. m.	30.6	8.21	4.70	....	....	...
	24 30 N.	81 55 W.	5 45 p. m.	31.6	8.23	4.60	....	....	...
July 31..	24 33 N.	81 48 W.	5 00 a. m.	30.6	8.21	3.96[2]	....	....	...
	24 33 N.	81 48 W.	12 40 p. m.	31.7	8.20	3.93	....	....	...
	24 33 N.	81 48 W.	3 30 p. m.	31.2	8.21	4.82	....	....	...
	24 20 N.	81 30 W.	6 30 p. m.	29.3	8.22	4.73	....	....	...
Aug. 1..	26 10 N.	79 40 W.	5 25 a. m.	29.2	8.21	4.58	....	....	...
	28 26 N.	79 20 W.	3 00 p. m.	29.8	8.21	4.5	9.9	25	3.3
Aug. 2..	31 13 N.	78 30 W.	5 20 a. m.	28.8	8.21	4.47	19.9	25	3.2
	33 40 N.	77 00 W.	3 00 p. m.	28.8	8.21	4.47	19.9	25	3.2
Aug. 3..	35 32 N.	75 04 W.	5 00 a. m.	21.0	8.10	5.45	17.6	22	3.5
	36 18 N.	75 20 W.	10 30 a. m.	25.4	8.19	5.25	17.1	22	3.0
	36 35 N.	75 22 W.	12 noon.	25.2	8.19	....	....	....	3.0
	36 52 N.	75 22 W.	1^h30^m p. m.	24.6	8.19	5.35	16.7	22	3.0
	37 09 N.	75 20 W.	3 00 p. m.	24.2	8.18	5.45	17.0	22	3.0
	37 32 N.	75 15 W.	5 00 p. m.	23.4	8.16	5.25	17.4	22	3.1
	38 25 N.	74 40 W.	9 00 p. m.	23.6	....	5.14	17.2	....	...
Aug. 4..	40 11 N.	73 55 W.	4 55 a. m.	23.0	8.23	5.25	16.5	22	2.5
	40 14 N.	73 55 W.	6 30 a. m.	22.6	8.22	....	16.5	....	2.6
	40 25 N.	73 55 W.	7 30 a. m.	21.5	8.04	....	....	....	4.2
	40 29 N.	73 55 W.	7 43 a. m.	19.3	8.88	....	15.4	21.5	4.5

[1] Inside Marquesas Lagoon. [2] Key West.

In figure 3 the stations from Key West to New York are marked by crosses and the appended numbers indicate the number of cubic centimeters of O_2 per liter. The track of the steamer during the night is marked by a black line and the track during the day is unmarked. Since the steamer had a speed through the water of about 12 knots, actual diurnal variations could not be determined. If, however, we assume that the Gulf Stream arises from a source of uniform composition, those observations in the axis of the stream should show the diurnal variations, except for the modifications due to local meteorological differences. Although *Trichosphærium* and other phytoplankton were present, and photosynthesis can occur at all depths less than 300 meters (light penetrating 1,000 meters), no marked or certain increase in O_2 in the sunshine was noted. At 5^h20^m a. m. August 2 there were 4.47 c.c. O_2 per liter and at 3 p. m. August 1 there was 4.50 c.c.; but no other pair of observations in the Gulf Stream shows a greater O_2

content in the afternoon than in the morning. Since oxygen is undoubtedly produced by these plants in the sunshine, the failure to observe an accumulation during the day probably lies in the fact that the temperature of the water rises about 1°, thus reducing the absorption coefficient for O_2 by 0.08 c.c. per liter and causing a passage of O_2 into the air. In contrast to this behavior of deep water, the O_2 concentration of the shallow water of Tortugas varied from 3 to 4.5 at about dawn to 4.5 to 7.0 at 3 p. m., the average maximum being shown in figure 4 opposite the station letters.

DISCUSSION.

Determinations of the pH from certain North Atlantic stations, table 8, made by Palitzsch (1912 *b*) compare favorably with those I have made in the Gulf Stream and in the deep water near Tortugas.

TABLE 8.

Depth, meters.	Sta. 80. 46° 17′ N. 7° 31′ W. 7 to 11 p.m. June.	Sta. 81. 41° 32′ N. 9° 32′ W. 4 to 6 p.m. June.	Sta. 84. 39° 22′ N. 9° 23′ W. 3 to 5 p.m. June.	Sta. 87. 37° 03′ N. 9° 15′ W. 1 to 5 p.m. June.	Sta. 91. 35° 53′ N. 7° 26′ W. 3 to 7 p.m. June.	Sta. 96. 35° 48′ N. 5° 58′ W. 9 to 11 a.m. June.	Sta. 97. 35° 50′ N. 5° 59′ W. 1 p.m. June.	Sta. 230. 35° 57′ N. 6° 00′ W. 11 a.m. September.
	pH.	*pH.*	*pH.*	*pH.*	*pH.*	*pH.*	*pH.*	*pH.*
0..	8.20	8.22	8.22	8.26	8.24	8.20	8.24	8.24
10..	....	....	8.13	....	....	....	....	....
25..	....	....	8.08	8.20	....	....	....	....
50..	....	8.18	....	....	8.18	....	....	8.20
75..	....	....	....	....	....	....	....	....
100..	8.08	8.13	....	8.13	8.13	8.20	8.20	....
150..	....	....	....	....	....	....	8.15	8.15
200..	8.08	....	....	8.10	8.08	8.13	8.13	8.13
300..	....	....	....	....	....	....	8.10	....
400..	8.06	8.04	....	....	8.04	....	8.08	....
500..	....	....	....	....	....	....	....	....
600..	....	....	....	8.06	7.98	....	8.07	....
700..	....	....	....	....	....	....	....	....
800..	8.04	8.01	....	8.04	7.95	....	....	....
900..	....	....	....	....	....	....	....	....
1,000..	8.01	8.01	....	8.01	7.98	....	....	....
1,200..	7.98	7.98	....	8.01	8.01	....	....	....
1,500..	7.95	7.95	....	8.06	....	....	....	....
2,000..	7.95	7.95	....	....	....	....	....	....

There is no increased amount of phytoplankton at Tortugas, but attached seaweed and symbiotic algæ and diatoms at the bottom cause the great diurnal change in O_2 concentration. On coral reefs the symbiotic algæ of corals and actinians are very effective, and in lagoons or other water which is not too agitated the symbiotic algæ of two species of the bottom medusa *Cassiopea* are a significant factor. One *Cassiopea xamachana* (11 cm. in diameter, weighing about 117 grams) in the sunlight gave out 1.9 c.c. O_2 per hour, whereas in the dark it absorbed 2.8 c.c. per hour, showing that 4.7 c.c. per hour was produced by photosynthesis, at 30°. In other words, the O_2 given out in the day is about two-thirds the amount used at night.

The production of oxygen by plants by photosynthesis depends on a number of factors. The data found in the literature are all in terms of leaf surface and not volume or weight. Haberlandt found the capacity of the chloroplasts of different plants to be about the same—*i. e.*, the rate of photosynthesis per unit area depended on the number of chloroplasts per unit area, as shown in table 9.

TABLE 9.

Plant.	Photosynthesis per unit area.	Chloroplasts per unit area.
Tropæolum majus...	100	100
Phaseolus multiflorus.	72	64
Ricinus communis...	118.5	120
Helianthus annuus...	124.5	122

With the same plant, photosynthesis depends on temperature, light, and CO_2 supply, and a lowering of any one of these factors may make it the limiting factor (law of minimum). Blackman showed that photosynthesis is about doubled by a rise of 10° in temperature. He showed this to be true of temperatures as high as 45°, provided the rate immediately after raising the temperature was calculated. With injurious temperatures the rate constantly falls, and the initial rate was estimated by extrapolation of the time curve.

Blackman and Smith, working on fresh-water plants, showed that photosynthesis is about doubled by a rise of 10° in temperature and varies directly with CO_2 tension in the water and illumination. Their maximal values, reduced to 30°, were about as follows, in cubic centimeters CO_2 per square centimeter per hour: *Fontinalis* 0.149, *Elodea* 0.093, *Ceratophyllum* 0.245.

Most of the determinations were made with *Elodea* and some leaves overlapped, so that the average thickness was a little more than that of one leaf. If we assume a thickness of 0.1 mm. and a specific gravity of unity, 9,300 c.c. of CO_2 per kilogram per hour were consumed in photosynthesis (about 10 per cent of this was re-formed by respiration of the plant during the day and the same amount during the night, so that perhaps 9,000 c.c. was converted into oxygen for the use of animals). This is a maximum value and would be reduced in case of marine plants by decrease in light due to clouds, inclination of the sun, and absorption by the water. A very rapid use of CO_2 by seaweed causes a crust of $CaCO_3$ to form on the surface, thus keeping out light and CO_2.

The number of cubic centimeters of O_2 used per hour per kilogram by various groups of animals is shown in table 10 (Krogh's average of various species at 20°, and Montuori's at 25° as compared with my determinations and calculations at 30°).

TABLE 10.

Group.	20°	25°	30°	Group.	20°	25°	30°
Jelly-fish	6	53	(12–75)	Cephalopods	115	179	(230–253)
Cassiopea	13	...	26, in the dark.	Other mollusks	58	28	(40–136)
Anthosoa	...	30	(42)	Crustacea	125	134	(190–250)
Worms	83	35	(50–166)	Tunicates	7	13	(14–18)
Starfish	...	31	(44)	Amphioxus	...	123	(174)
Sea-urchins	...	19	(27)	Fish	110	177	590 (220–250)
Sea-cucumbers	...	14	(20)	Reptiles	92	...	(184)

The discrepancies are due to differences in size, activity, and species. The calculations (in parentheses) were made on the assumption that the respiration at 30° is double that at 20° and 1.414 times that at 25°. A kilogram of the small fish I studied would use up all of the O_2 in 3,600 liters of the sea-water of the Gulf Stream in 24 hours. The respiration of the very active animals studied by others is about half as rapid and there is great variation with the species in sluggish animals.

With 1 kg. of fish in 300,000 liters of warm sea-water we should be able to detect a distinct fall in O_2 concentration during the night. In order to attain this effect there need be organisms, the equivalent of 1 kg. of fish, to 10 square meters of bottom in water 30 meters deep.

It will be seen from the foregoing sections that the changes in the sea that were considered are very complicated. If the dirunal curves are plotted they show secondary notches. It was thought probable that the secondary notches in the diurnal curves of the sea-water at station A might be due to horizontal and vertical currents and eddies. In order to exclude some of these currents the diurnal variation of a model lagoon was studied. A glass tank about 4 by 6 by 4 feet was placed in the open air and filled with sea-water and a few cassiopeas placed on the bottom. Convection currents stirred up the water in the tank, but there were necessarily no extensive horizontal currents over bottoms of different character and depth. The curves of the diurnal variations were smooth—that is, without secondary notches. The extremes (maxima and minima) of the curves were at about 5^h40^m a. m. and 4 p. m. The morning extreme was a little delayed, owing to partial shading of the tank for a short time, before and after sunrise, but there was a corresponding shading before sunset and therefore the curves were not skewed. The smooth curves obtained with the tank indicate that the notches in the diurnal curves at station A are due to currents in the water and not to technical errors. On partially cloudy days it seems possible for clouds to make notches in the diurnal curves, and sudden squalls have a similar effect.

On July 10, 19, 20, 21, 22, 23, 24, and 25 very frequent determinations were made, so that the notches in the curves could be studied. With the omission of these small notches, the diurnal curves are plotted in figure 6. It may be observed that there was a general upward drift in temperature during July of about 2°. As a consequence of rise in temperature and increased cloudiness, there was a fall in the oxygen concentration in the sea-water during July. July 10 was an unusually calm day (up to 9 p. m., when a brief squall appeared) and shows great extremes in all determinations. Contrasted with this are the windy days, July 13, 14, 15, and 16, during which the oxygen curves were very much flattened out. This shows that the oxygen content of the sea-water is somewhat influenced by the atmosphere. In figure 3 it may be observed that the oxygen content of the water north of Hatteras is increased with decreasing temperature and increasing absorption-coefficient, which is another demonstration that the oxygen content of the water is influenced by the air. On the other hand, shallow water is not nearly in equilibrium with the air in regard to oxygen. At

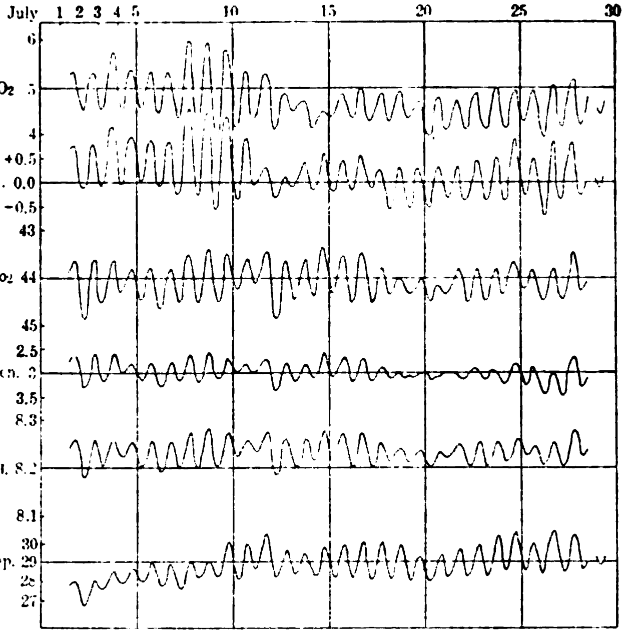


FIG. 6.—Diurnal variation curves of sea-water from station A, Tortugas, during July, 1917. The date is marked on the right-hand border of the day interval, or where omitted it may be determined by counting the waves of the curve. The trough of the wave represents the early morning and the crest of the wave represents the early afternoon. The top curve represents the number of cubic centimeters O_2 per liter; the second curve, the excess or deficiency of saturation; the third curve, the number of cubic centimeters CO_2 per liter; the fourth curve, CO_2 tension; fifth, pH; and sixth, temperature.

stations B and H samples of water were obtained which were 20 per cent undersaturated and 69 per cent oversaturated respectively.

Contrasted with the behavior of oxygen, no certain effect of the atmosphere on the CO_2 of the water as indicated by the pH was observed. At station A the oxygen curves were flattened out on July 13–15, due to high winds, but no flattening is observed on the pH or CO_2 curves. On going north into colder waters no fall in pH was noted, except on a very cloudy day at the mouth of the Chesapeake Bay and in the polluted water off New York Harbor, neither of which can be considered normal conditions. Palitzsch, however, noted that pH fell from 8.08 to 8.13, with more extreme drop in temperature in the North Atlantic and North Sea. This is not due to a difference in standards, since he found the pH of the Altantic at about 41° north latitude and of the Bay of Biscay to be 8.22 and 8.25 respectively.

No local effect of the CO_2 tension of the sea on the CO_2 of the air was observed. The wind reaching Tortugas blew over the Florida Keys and alternate areas of water of CO_2 tension$=3$ to 3.5 in the morning and 2 to 3 in the afternoon. The CO_2 of the air varied from 2.8 to 3.5 without any relation to time of day. Part of this variation may have been merely the expression of technical errors, but a large number of duplicate experiments, and of experiments designed to show the limit of error, failed to support the idea that the variations were entirely due to technical errors. The CO_2 of the air was determined colorimetrically with 24 mm. sealed tubes containing the standards. The objection might be raised that the expanse of water of the same CO_2 tension was not great enough to affect the air and that considerable time was necessary to effect a change, and while one body of air was passing over the sea, both morning and afternoon variations in the water affected it alternately with neutralization of effects. The variations from day to day might have been due to slight shiftings of the wind and also to vertical currents in the air. In order to avoid such objections, Dr. A. G. Mayer made determinations over the Pacific Ocean. The standard tubes that he used were only 10 mm. in diameter, but the bicarbonate solution was kept in a gold-lined Jena flask and had not changed when tested on his return. The absolute standards may have been different from those used at Tortugas, but the method was adequate to show differences, provided equilibrium was always reached. The determinations were taken at noon each day, except where otherwise stated. The results of my calculations from his data are given in table 11.

From table 11 there probably may not be recognized any correlation between CO_2 tension of the sea and of the air. The cause for this may be that the apparatus was too crude or that exchange between air and sea is entirely masked by currents in the air. If we regard the air as sufficiently circulated to be of practically uniform composition, the changes in barometric pressure would not cause significant changes in CO_2 tension and the latter might be considered constant. If there were sufficient exchange between sea and air the CO_2 tension of the sea would be practically constant. In table 11, the sea-water examined by Dr. Mayer on the trip from San Francisco to Hawaii (if we except the first station, where the water was polluted from land drainage and came in an insensitive region of the thymolsulfonphthalein tubes) had an almost uniform CO_2 tension, although the temperature rose 6.2°, thus necessitating in total CO_2 a fall of 1.3 c.c. per liter and rise in pH of 0.08. This may not, however, have been entirely due to exchange with the air *in situ*, but partly to the remote history of the water. Palitzsch has shown that the pH of the open sea decreases as the depth increases. It is well known that temperature decreases as the depth increases, and the two factors would have antagonistic effects on the CO_2 tension and tend to keep it constant. The reason for this stratification of the water may be that each stratum of water reached a certain temperature and CO_2 tension while at the surface, and the temperature effect on the density caused the superposition of the strata. After being buried out of reach of surface currents, the temperature and total CO_2 change very slowly and hence the CO_2

TABLE 11.

Date.	Latitude.	Longitude.	Bar.	CO_2 tension air.	CO_2 tension sea.	pH sea 20.	Temperature of sea.	Salinity.
Feb. 21..	36° 05′ N..	128° 38′ W..	756	3.1	3.1	8.05	12.3	.....
22..	33 31′ N..	135 09′ W..	758	3.5	2.4	8.17	15.4	.....
23..	30 53′ N..	140 53 W..	750	2.6	2.4	8.20	17.6	.....
24..	27 49 N..	146 28 W..	762	2.4	2.4	8.23	20.1	.....
25..	24 22′ N..	152 19 W..	763	3.0	2.4	8.25	21.6	.....
27..	17 52 N..	159 13 W..	762	3.0	...	....	....	.....
28..	12 10 N..	161 17 W..	761	3.1	...	....	....	.....
Mar. 1..	06 35 N..	163 21 W..	759	3.5	...	....	....	.....
2..	01 07 N..	165 30 W..	757	3.0	...	....	....	.....
3..	04 34 S...	167 23 W..	757	3.5	...	....	....	.....
4..	10 14 S...	169 14 W..	758	2.4	...	....	....	.....
Apr. 19..	10 15 S...	169 03 W..	757	3.0	2.8	8.24	27.8	35.10
20..	05 10 S...	167 00 W..	756	4.1	3.6	8.15	26.8	35.41
21*a*.	00 00 ...	165 05 W..	756	2.9	3.7	8.12	25.0	35.26
22..	05 42 N..	163 12 W..	756	4.1	4.1	8.10	25.9	35.05
23..	11 19 N..	161 05 W..	758	3.0	2.7	8.22	25.4	34.58
23*b*.	12 29 N..	160 39 W..	...	...	2.7	8.23	25.5	.....
24..	17 03 N..	159 23 W..	760	2.9	2.8	8.21	24.5	34.58
25*c*.	21 00 N..	157 50 W..	...	...	2.7	8.21	23.8	.....
25*d*.	21 10 N..	137 40 W..	...	...	2.8	8.20	23.9	34.94
26..	23 51 N..	153 44 W..	760	3.6	2.7	8.21	23.6	34.79
27..	27 18 N..	147 52 W..	760	3.5	2.5	8.23	21.6	35.05
28..	30 29 N..	141 50 W..	761	4.0	2.5	8.20	19.8	35.05
29..	33 39 N..	135 34 W..	762	3.1	3.1	8.10	16.6	33.89
30..	36 00 N..	128 59 W..	762	3.1	2.8	8.10	14.3	33.17
May 1*e*.	37 50 N..	123 25 W..	...	...	5.2	7.85	10.5	33.33

a $11^{h}00^{m}$ a. m. *b* $5^{h}00^{m}$ p. m. *c* $5^{h}00^{m}$ a. m. *d* $6^{h}00^{m}$ p. m. *e* $7^{h}30^{m}$ a. m.

tension changes very slowly and may be practically the same when it reaches the surface the second time as when it left the surface the first time. Palitzsch's data are shown in table 8.

It is not intended to create the impression that the air regulates the CO_2 tension of the sea. There is 20 to 30 times as much total CO_2 in the sea as in the air. If there were no CO_2 in the air and this condition could be maintained by some external agent, all of the CO_2 in the sea would pass into the air, thus reducing the carbonates and bicarbonates to hydrates. In other words, the total CO_2 of the sea is available for regulating the CO_2 of the air. From the available data, however, it appears that the partial pressure of CO_2 in the air at sea-level is less than the average CO_2 tension of the sea. The reason for this anomaly is as follows: Part of the carbon fixed by photosynthesis at depths of less than 300 meters gravitates to greater depths and is oxidized, thus increasing the CO_2 content of deep water. Some of this deep water is constantly being carried to the surface by vertical currents, but on reaching the light the CO_2 is reduced again by photosynthesis and the original state of affairs is regained. Thus the sunlight interposes a barrier between the air and the main body of sea-water, so that the air contains less CO_2 than it would if the sea were more rapidly stirred.

Thus photosynthesis has an effect on the CO_2 of the air that is not entirely counteracted by the respiration of animals, because animals live not only at the surface but at all depths. The amount of photosynthesis must affect the CO_2 content of the air, but so many factors affect photosynthesis that probably no one factor is the limiting factor for the whole surface of the sea. Photosynthesis varies directly with both CO_2 tension and illumination, and either of these might become a limiting factor under certain conditions. There is an optimum pH range for plants, and since the temperature of the sea-surface as a whole is dynamically constant, there might be constructed an optimum CO_2-tension map of the sea. But the range of pH compatible with the life of seaweed is rather broad and may be different for different species and has not been worked out for the whole life-history of a single species, and besides, there may be some other limiting factor. It seems probable that the limiting factors are different in cold and warm seas. Photosynthesis is more than doubled by a rise of 10° in temperature, but the number of grams of plant tissue per square meter of sea surface is less in warm seas than in cold seas. The limiting factor in very cold seas may be temperature. The limiting factor at Tortugas may be fixed nitrogen, since I found less than 0.02 mg. of fixed nitrogen per liter of sea-water. There is a more abundant growth of eel-grass on the west side of Loggerhead Key than on the east, and sewage and garbage contaminates the water of the west side. On the other hand, the water of the west side is less agitated by the wind. At the Marquesas, fixed nitrogen is washed into the sea from decaying organic matter on shore. There is an abundant growth of eel-grass in shallow water around Marquesas and in the lagoon where the water is not extremely shallow or perhaps disappears at low tide.

In the shallow pool between Bush Key and Long Key, Tortugas, blocked off from the sea except at extreme high tide by sand-bars thrown up in a recent hurricane, there is a dense growth of seaweed, which probably gets its fixed nitrogen from the adjacent shores. It seems probable that the fixed nitrogen formed by electric discharges, decomposition of organisms, and nitrifying bacteria that finds its way to Tortugas is so reduced by the action of Drew's bacilli as to be the limiting factor for the growth of seaweed. At any rate, the amount of phytoplankton per unit volume of sea-water is much smaller than in cold seas. Only on the bottom, and especially along shores where the plants may have access to fixed nitrogen (from the decay of organisms) before Drew's bacilli have had time to decompose it, do we find an abundant plant growth.

The limiting factor for animals seems to be food, but under some unusual conditions oxygen might become the limiting factor. Fish in an aquarium open to the air, and with a constant stream of oxygenated sea-water flowing in, suffocate if the stream of water is not sufficiently rapid. The same is true of sponges and other animals. If we take the lowest oxygen concentration found right at the sea surface, 1 kg. of fish would use all of the oxygen in 4,300 liters of sea-water in 24 hours and would show symptoms of altered metabolism before it was used up. Oxygen diffuses into the water from the air very slowly, and the fact that fish can come to the surface does not help them much. It seems improbable that fish would congregate in such numbers as to suffocate. On the other hand, dinoflagellates sometimes multiply or congregate in such numbers as to die and make the water foul. In the summer of 1907 I observed such swarms of *Gonyaulax polyedra* in the Pacific Ocean off La Jolla, California, that the water was red by day and turned to fire at night. The infected water welled up in spots which grew larger until nearly all of the water was infected. In about 3 days the water stunk and some dead fish were cast on shore by the waves. The death of large numbers of fish from unknown causes are reported by Taylor. It is not intended to imply that these were due to lack of oxygen, but the oxygen content of the water was not investigated and no satisfactory explanation was found.

No direct determinations showing absence of oxygen in sea-water have come to my notice, but H_2S in the water is destroyed by oxygen and the presence of H_2S indicates oxygen-lack. In some Scandinavian fjords H_2S sometimes rises to within 4 feet of the surface and kills the oysters in "oyster polls." In the Black Sea H_2S appears at a depth of 180 meters, according to Palitzsch.

PART II.

RELATION OF OXYGEN TENSION AND ACTIVITY OF THE NERVOUS SYSTEM TO METABOLISM IN CASSIOPEA.*

Henze (1910*a*) observed that sea-anemones use less oxygen when less is present in the sea-water, but interpreted this as due to the time required for diffusion into the animal. That is to say, he supposed that all of the cells were not supplied with oxygen when there was little in the sea-water. If oxygen was entirely absent in some of the cells, the decreased oxidation may have been merely the expression of the lesser number of cells taking part in the metabolism. Burrows showed that tissue cells require a certain oxygen tension for growth. Verzar showed that the cat's heart is slowed by reducing the oxygen, and Loeb and Wasteneys found that the heart-beat of *Fundulus* embryos may be slowed by reducing the oxygen. The growth of *Fundulus* may be suspended by lack of oxygen and may be slowed by diminishing the oxygen. Johansen and Krogh found that plaice-fish eggs develop slower when the oxygen tension is reduced.

The calorimeter consisted of either an 850 c.c. or a 900 c.c. Dewar flask (thermos bottle) inclosed in an air-tight container, which was immersed in water that was maintained at the same temperature as the water in the flask, within 0.003°. The technical difficulties were met as follows: Two Beckmann thermometers were adjusted and compared over the range of temperatures of the experiments and fitted with reading-lenses to estimate down to 0.001°. A large tank of sea-water was brought to 30° (which was about the temperature of the air) and its pH and O_2 and CO_2 content determined. A cassiopea was introduced into some of this sea-water that was dipped out into a jar. The thermos bottle, stopper, and thermometers were immersed in the large tank until they reached the temperature of the water. The cassiopea was transferred to the thermos bottle and a perforated cork stopper was inserted, with the exclusion of air-bubbles. One Beckmann thermometer was inserted through the perforation in the stopper and the other suspended in the tank, near the middle of the thermos bottle. A small hole, remaining in the stopper for exit of displaced water, was closed with wax. The pulsations of the cassiopea stirred the water inside the thermos bottle, and the water in the tank was mechanically stirred and was kept at the same temperature as that inside the thermos bottle by additions of small portions of warmer or colder water as required. The light was excluded by the silvering and coverings of the thermos bottle, but in some experiments in which a 900 c.c. glass jar with ground-glass cover was used in place of the thermos bottle, the light could be excluded by darkening the tank, so as to prevent photosynthesis in the symbiotic plant cells. Time was measured by means of a stop-watch and a clock. The same cassiopea was used in a series of experiments.

---

*The content of this section was published as a separate paper in Jour. Biol. Chem., 1917, xxxii, 275.

The oxygen in the sea-water was determined by the Winkler method, which can be corrected for the small error due to a slight amount of organic matter given out by *Cassiopea*, and it was thought impracticable to use the complicated method of Shutzenberger and Risler (Henze, 1910*b*). A 250 c.c. glass-stoppered bottle was weighed empty and full of distilled water at a known temperature, in order to standardize its volume. It was fitted with a double-bored rubber stopper and a long and a short glass tube with rubber connections. The bottle was filled with mercury and the long glass tube was sucked full of the water to be analyzed and the stopper inserted. By inverting the bottle the sea-water was siphoned into it, when the rubber stopper was removed and the glass stopper inserted. The glass stopper was lifted and 1 c.c. of alkaline KI solution and 1 c.c. of 40 per cent $MnCl_2$ solution introduced (correction being made for this in the O_2 calculation) and the stopper was inserted. Colloidal membranes formed about the drops of alkali and violent shaking was necessary to break them. After the precipitate had settled, 2 c.c. of concentrated HCl were added and the stopper inserted and the bottle shaken. Its contents were transferred to a flask and titrated with 0.01 N sodium thiosulphate solution until the yellow color disappeared; then starch solution was added and the titration continued until the blue color disappeared; then the water was poured into the bottle and back into the flask and titrated until the blue color disappeared. The calculation was as follows:

$$\frac{0.056 \times \text{c.c. thiosulphate}}{\text{capacity of bottle} - 2} = \text{c.c. of oxygen per liter of sea-water}$$

and this quotient multiplied by 0.9 equals cubic centimeters of oxygen in calorimeter (on the assumption that the concentration of oxygen in the cassiopea was the same as that in the sea-water).

In order to reduce the carbonate content, the alkaline KI solution was made fresh every few days from two stock solutions. Carbonate-free NaOH solution was made by dissolving 100 grams of NaOH in 100 c.c. H_2O in a glass-stoppered bottle and pipetting off after the carbonate had settled. One part of this was mixed with one part of 20 per cent KI solution before being used. NaOH made from metallic sodium contained a trace of nitrite and NaOH purified by alcohol contained but little more. This nitrite causes no error if the titration is quickly made immediately after adding the acid, but the contents of the flask slowly turn blue for hours after the end-point has been reached. If the acidity is greatly increased, however, the nitrite causes an appreciable error in the titration.

The $MnCl_2$ solution contained a trace of $Mn(OH)_2$, but this was removed by decantation. The thiosulphate was dissolved in CO_2-free water and kept in an automatic burette with soda-lime tubes, and standardized with pure iodine. The starch solution was allowed to settle and only the clear upper portion used. When dextrins appeared in it a fresh solution was prepared.

Although pH determinations and CO_2 calculations were made in all experiments, it was found that the oxygen absorbed could be determined much more accurately than the CO_2 given out, and the CO_2 determinations are not listed with the experiments, but are collected together in the form of respiratory quotients. The respiratory quotients were 0.7, 0.74, 0.76, 0.84, 0.85, 0.86, 0.88, 0.9, 0.91, 0.92, 0.97, 0.99, 1, 1.02, 1.03, 1.1, 1.15, 1.2. It was impossible to tell whether the variation is due entirely to errors in the CO_2 determinations or whether the respiratory quotient varied. It is improbable, however, that respiratory quotients of 1.2 existed for even short periods of time, and these at least may be considered due to technical errors. According to Mayer (1914), *Cassiopea* lives on animal food exclusively and does not absorb carbohydrates from its symbiotic algæ, since it starves as rapidly in the light as in the dark. It may, however, get some carbohydrates from its animal food, or from glycoproteins during starvation. In this connection it may be of interest to note that *Cassiopea* secretes a mucin-like substance. Since the error in estimating CO_2 production may be 30 per cent in half-hour experiments with small cassiopeas, it is convenient to assume that the respiratory quotient is constant and is about 0.95, which is also the average found by Vernon for the hardier species of jelly-fish, on which he made most of his determinations.

The oxygen consumption is about doubled when the temperature is raised from 20° to 30°, and from results on other animals is probably an exponential function of the difference in temperature. Harvey (see Mayer, 1917) found the velocity of the nerve-impulse in *Cassiopea* to be a linear function of the temperature and to increase about 64 per cent on raising the temperature from 23° to 33°. I found the activity of the ganglia (rhopalia) in inducing pulsations of the umbrella to be about doubled with rise from 20° to 30° in temperature. The data are given in table 12.

TABLE 12.

O_2 consumption.		Temp. coeff. for 10° rise.	Pulsations per second.	
20°	30°		20°	30°
0.698	1.54	2.21	....	....
0.57	1.05	1.84	0.27	0.55

TABLE 13.

pH.	O_2 concentration.	O_2 used.
7.50	4.40	1.85
7.53	4.50	1.95
8.24	4.30	2.79
8.24	4.50	2.87
8.24	4.55	2.43
8.38	4.40	2.07
8.52	3.90	2.28
8.72	3.16	2.59

These experiments show the necessity of accurate temperature control, and in all of the other experiments in this paper the temperature was measured to the nearest tenth of a degree and maintained to within 0.2° of 30°.

The sea surface is at about the optimum pH for metabolism (usually 8.1 to 8.3), but the variation in metabolism with variation of pH within the range studied is very slight, as shown by table 13 (diameter of cassiopea = 10 cm.).

These variations in oxidation may be due to experimental errors and variation in O_2 concentration, except the first two, which show a slight lowering of oxidation when the pH is reduced to about 7.5.

In comparing the rise in temperature in the calorimeter with that calculated from the O_2 consumption, the assumptions were made that the respiratory quotient was 0.95, and that a certain mixture of proteins, fats, and carbohydrates was burned, giving 6 gram-calories per cubic centimeter of CO_2, as in table 14.

TABLE 14.

O_2	CO_2	Determined.	Calculated.
		gr. cal.	*gr. cal.*
2.18	2.07	12.6	12.4
2.54	2.41	16.2	14.5

In these experiments it was assumed that there was no loss of heat, although some heat must have passed into the thermometer bulb and glass lining of the thermos bottle. The specific heat of the sea-water and the cassiopea was taken as unity because the determinations were not accurate enough to warrant the application of small corrections. The experiments had to continue for 2 hours in order to obtain an accurately measurable rise in temperature, and the tedium of keeping the tank at the same temperature as the calorimeter necessitated the substitution of indirect calorimetry in the remainder of the experiments.

Some preliminary experiments to show the effect of O_2 tension are shown in table 15. Since the temperature was constant, the O_2 tension is proportional to O_2 concentration.

Average O_2 concentration	11.9	1.91	::	3.36	1.55
O_2 consumption	2.6	2.38	::	2.26	1.65

In performing these experiments, a number of possible sources of error were thought of and it was decided to make a more detailed study of the metabolism of *Cassiopea* before returning to the subject. The chief danger of error was in prolonging the experiment until all of the O_2 was used up. It was found that *Cassiopea* could live more than 7 hours without oxygen, in which case no measurable quantity of CO_2 was produced. Vernon observed practically no increase in the respiratory quotient of jelly-fish correlated with oxygen-want, whereas the respiratory quotient of fishes increased under these conditions.

In order to determine whether the rate of oxidation depends on the oxygen tension, it is desirable to know something about the oxygen tension inside the living cells. In other words, the transfer of oxygen to the cells must be facilitated as much as possible if we are to judge anything about the tension of O_2 within them from that in the sea-water.

This could be approximated by agitating free cells or a single layer of cells with the water or circulating the water over a single layer of cells. When using free cells, some are liable to injury and more or less disintegration, thus interfering with the titrations, but, notwithstanding the criticism of Heilbrunn, comparative results may be obtained (McClendon and Mitchell). *Cassiopea* was chosen because the cells are spread in thin layers on the surface of a mesoglea which will be shown to use practically no oxygen. The pulsations of a cassiopea bring currents of water over the cell-layers, so that diffusion is necessary only for a minute distance. The error due to this diffusion would be large only when the O_2 tension is very small. By skillful manipulation, the mucous secretion may be prevented from increasing or leaving the surface of the cassiopea.

Evidence that oxidation is confined to the cell-layers is apparent in the fact that oxidation is not proportional to the volume but to the surface. It would be practically impossible to measure the surface, but since the individuals are practically of the same shape, the surface is proportional to the square of the diameter. Since *Cassiopea* is elastic, the diameter was always measured under the same conditions, *i. e.*, resting on a glass plate, with the exumbrella in contact with the glass (and the average of 2 diameters at right angles to one another taken). Some rough preliminary determinations showed the O_2 consumption in cubic centimeters per hour to be about 0.023 time the square of the diameter in centimeters, as shown in table 15. Very small cassiopeas used more O_2 than calculated from the formula (an anomaly which is correlated with more rapid pulsations). A cassiopea 3.5 cm. in diameter pulsated once a second, whereas one 10 cm. in diameter pulsated 0.3 time per second. In order to compare experiments on *Cassiopea* where the weight is recorded, it is convenient to know that the diameter is cm. $= 2.25^3$ weight in grams.

TABLE 15.

Diameter.	O_2 per hour.
cm.	
3.5	0.4
7	1.4
8.5	1.17
9.5	2.16
10	2.7
11.5	2.78

Table 16 gives the respiration-rate under different conditions, except that the temperature is always 30°.

In this table the pH and O_2 per liter at the beginning of the experiment are given; the pH was about 0.09, while the O_2 was 1.5 c.c. lower at the end of each experiment. The average O_2 during each experiment influences the O_2 used per hour, but apparently no difference in the quotient of the O_2 used per hour divided by the square of the diameter can be correlated with difference in size. Using greater extremes of size, however, the quotient seems to decrease as the diameter increases, and therefore extreme sizes were usually avoided after this was discovered.

All of the experiments were made under conditions of starvation, and hence the cassiopea used its own substance as a source of energy. Starvation can hardly be considered a pathological process in *Cassiopea*, however, since it may remain alive for months without food, constantly decreasing in weight until it almost disappears before death. Mayer (1914) determined the loss in weight as about 5.6 per cent per day at about 30°, although no thermostat was used. If y is the weight at any moment and w is the weight when starvation commenced and n is the number of days of starvation,

$$y = w(1 - 0.056)^n$$

Table 16.

Diameter.	O_2 per hour sq. dia.	O_2 per liter.	pH.	O_2 per hour.
7	0.0200	4.5	8.20	1.3
8	.0187	4.2	8.22	1.26
8.5	.0152	3.32	8.22	1.09
10	.0287	4.5	8.24	2.87
10	.0279	4.3	8.24	2.79
10	.0243	4.55	8.24	2.43
10	.0185	4.4	7.50	1.85
10	.0195	4 5	7.53	1.95
10	.0207	4.4	8.37	2.07
10	.0228	3.9	8.52	2.28
10	.0259	3.16	8.72	2.59
11	.0230	3.54	8.17	2.79
11	.0200	1.8	8.40	2.43
11	.0160	1.57	8.02	1.93
11	.0320	7.2	8.47	3.84
11	.0400	7.44	8.46	4.87

Since I found the diameter to be 2.25 times the cube root of the weight, if the weight were 100 grams, the diameter would be 10.45 cm. The O_2 consumed during one day would be about 0.023 time the square of the diameter times 24 = about 60 c.c. O_2 absorbed and 57 c.c. CO_2 given out. If we assume that protein was burned and that 5.9 gram-calories correspond to 1 c.c. CO_2, the metabolism would equal 336 gm. cal. for the day. If we assume that a certain mixture of proteins, fats, and carbohydrates was burned and 6 calories correspond to 1 c.c. of CO_2, the metabolism would equal 342 calories per day.

Since I have shown that the metabolism is proportional to the surface and Mayer has shown that the loss in weight is proportional to the volume (weight), the composition of the cassiopea must change during starvation. In other words, it loses weight faster than it burns protein (or other organic matter), and hence the concentration of the protein must increase. Mayer (1914) found the cellular layer did not decrease in thickness during starvation, and Hatai found the percentage of nitrogen to the total body-weight increases during starvation and is also greater in small than in large, well-nourished cassiopeas. Therefore in attempting to calculate the metabolism from the loss in body-substance, we should make it proportional to the loss in surface rather than loss in volume, since the loss in living matter seems to be proportional to the loss in surface and the cassiopea seems to have no other important store of food than its own protoplasm, the mesoglea apparently functioning chiefly as a skeleton.

Since the surface is proportional to the two-thirds power of the volume, we may assume that the protein is proportional to the two-thirds power of the weight (the density remaining practically constant). The protein equals 5.16 per cent of the two-thirds power of the weight (calculated from Hatai's data on the assumption that protein is 16 per cent N). The weight at the beginning of starvation was 100 grams and the protein 1.107 grams; at the end of one day the weight was 94.4 and the protein 1.07, being a loss of 37 mg. of protein. If we assume that 1 mg. of protein is equivalent to 4.4 cal., the metabolism the first day would be 163 calories, although I found it to be 336 to 342 calories.

Although these calculations are only approximate, since starvation is a little greater the first day than calculated by the formula, this great difference indicates that the burning of protein does not account for all of the heat. Since living cells contain lipoids or lipo-proteins and carbohydrates or glyco-proteins, it seems probable that proteins, carbohydrates, and lipoids are burned. The mesoglœa has not been analyzed separately, but is largely sea-water, with possibly a trace of glyco-protein. It probably has little calorific value, since the use of a store of food would cause a relatively greater metabolism in large starving cassiopeas than was actually observed.

Since the lining of the alimentary tract is not at the surface of the cassiopea and O_2 must diffuse through at least a millimeter of tissue to get to it, it was decided to pull off the manubrium and study the metabolism of the umbrella. The umbrella is disk-shaped, is covered on both sides by epithelium, and pulsates, thus circulating the water. The wound made by removal of the manubrium is of small area and is covered by an epithelium within a few hours, and the umbrella will live as long as a starving cassiopea. Some rough determinations indicate that the respiration of the umbrella is only about a fourth as great as that of the whole cassiopea. Table 17 records the measurements on 3 cassiopeas and on their umbrellas after removal.

TABLE 17.

Diameter.	O_2 per hour $\div$ (diameter)^2.		Pulsations per second.
	Cassiopea.	Umbrella.	
cm.			
3.5	0.033	0.0075	0.94
9.5	.021	.0041	.58
11.5	.030	.0075	.63

Since the respiration is influenced by the muscular activity or pulsation-rate, and the latter is not constant, it was decided to remove the ganglia (rhopalia) that induce the pulsations and start a continuous contraction-wave running around the subumbrella (the middle third of which has no neuro-muscular tissue, Mayer, 1908). The rhopalia were cut out by means of a cork-borer and the wave started by electrical stimulation. It was noticed, however, that the contraction-wave, apparently constant for short intervals of time, changed more rapidly at first and then more slowly, but never became absolutely constant, the change being perhaps associated with shrinkage of the umbrella. The effect of shortening and stretching on the contraction-wave was therefore studied. The rate of the contraction-wave depends on the rate of the nerve-impulse around the circuit of the nerve-muscle layer, but does not depend solely on the rate in the neuraxon, since there are numerous synapses, and furthermore, the path of the impulse is zigzag. Prof. L. R. Cary kindly showed me a stained preparation of the nervous network of the subumbrella. Concentric rings cut from the umbrella are capable of maintaining a trapped wave for some time, but if the ring is too narrow, the wave can not be started or soon ceases after being started. Trapped waves can be started in 2 or 3 concentric rings cut from the umbrella and the wave revolves about the inner ring more often per second than about the outer ring, but the revolutions per second are not in exact inverse proportion to the mean diameters of the rings or to the diameters of the inside tracks or holes in the rings. One subumbrella, 11.5 cm. in diameter, was cut into two rings and waves were trapped in them. The wave in the inner ring made 2.5 revolutions per second and the wave in the outer ring made 2 revolutions per second.

The uncertainty as to the length of the pace-making circuit that the nerve-impulse takes around the ring may be avoided by stretching the inner edge of the ring until it is of the same diameter as the outer, thus transforming the ring into a cylinder or belt. Such a ring can be stretched further and behaves in a strikingly reversible manner. Since Mayer (1917) has shown that the rate of nerve-conduction in *Cassiopea* depends on temperature and electric conductivity of the sea-water, it should be noted that all the experiments in this paper, unless otherwise stated, were made in sea-water of 30° and Cl = 20. Mayer found a variation of only about 2.5 per cent over the range of pH = 5.6 to 8.26, and this variation includes experimental errors and changes due to unknown causes. In the present experiments the pH was about 8.2 unless otherwise stated. The only difficulty in estimating the rate of the contraction-wave arose from the fact that the rate is 1 to 5 per cent faster in the ring that has just been stretched than in the ring that has just been relaxed, depending on the degree of recent stretching or relaxation. If, however, the circumference of the ring is allowed to remain constant for 5 minutes after each short step of stretching or relaxation, the rate will approximate a mean value.

This behavior of the ring may be regarded as a form of hysteresis, since the number of revolutions per second of the contraction-wave tend to remain constant immediately after stretching or relaxation. I do not see how this can be explained on the assumption that the stretching of the neuraxon is the only factor, and it is significant to note that Carlson records no such hysteresis in the stretched nerve of the slug, where synapses are less numerous or entirely absent. One explanation of the increased rate immediately after stretching might be the thinning of the plasma membrane of the neuraxon, the regeneration in thickness taking appreciable time. Another suggestion is made that the immediate effect of stretching is increase in length of the neuraxon, but that this may be proportionately less than the increase in length of the strip of tissue, since the nerve-paths may be straightened, and that the apparent hysteresis is due to the possibility that after the nerve is stretched synapses gradually open, due to the tension, until the nerve-paths are proportionately as zigzagged as at the start. This supposition may similarly be applied to the fact that by prodding a cassiopea (having a trapped wave) with a stick, the number of revolutions of the wave per second is reduced, although no apparent increase in length of the conducting-path remains. The local stretching of the subumbrella with the stick might break some of the synapses.

Such complicated suppositions are not very useful, however, since they are not easily tested. A more probable hypothesis is given below in connection with the amplitude of the contraction-wave. The significant fact is that (ignoring the period of readjustment) the ring may be stretched until the circumference is increased 72 per cent with practically no change in rate (millimeters per second), although in order to accomplish this the number of revolutions per second or passages of the wave through the same tissue may be reduced 46 per cent. This is analogous to the effect of stretching a metallic wire on the passage of an electric current through it, with the difference that the process is completely reversible in *Cassiopea* after an increase of 84 per cent in length due to stretching. The results on two rings are shown in table 18, the rate being given in millimeters per second.

TABLE 18.

Circumference....	223	263	283	286	306	326	346	366	386	406	426	446	466	486	506	526
Rate	372	413	430	379	390	399	410	414	407	403	391	377	368	360	352	342

If the rate of wave propagation is the same, we would expect that in umbrellas of cassiopeas of different sizes the number of revolutions of the wave per second would be inversely proportional to the diameter. The diameter is measured before the wave is started and there is a progressive shrinkage in the diameter, due to starvation and decrease in volume, tension of the regenerating tissue after removal of the rhopalia, and increased tonus of the musculature (sometimes transforming the disk into a cup-shape). This decrease in the diameter is associated with increase in revolutions per second, but agitation decreases the revolutions per second. It is therefore necessary to make the determinations under the same conditions for strictly comparative results. The determinations in table 19 were rough, but serve to indicate the general features.

TABLE 19.

Diameter.	Revolutions of wave per second.		
	Immediately.	After 24 hours.	After prodding with a stick.
cm.			
9.75	2.38	2.50	....
10.50	1.79	2.17	2.04
10.50	1.75	2.17	2.04
10.75	1.75	2.13	....
11.00	2.38	2.08	....
11.50	1.72	2.22	....
11.50	1.79	2.22	....
12.50	1.67	1.72	1.66
12.80	1.67	2.00	....
13.50	1.61	1.67	1.40
13.50	1.61	1.79	1.52
14.00	1.67	1.79	....

$\bar{x}$ = 1.82 ± .27 2.03 ± .25

A comparison of these experiments with those on the actual velocity of the wave shows the circumference of the potential pace-making circuit to be about 1.8 times the diameter.

If the contraction-wave is stopped by pressure and after a rest of some minutes or hours is started again, it is slower than just before stopping, but if it is started again as quickly as possible after stoppage the rate is the same. This effect of a rest may be associated with nutrition or recovery from fatigue, since the amplitude of the contraction-wave is greater in the rested umbrella, although the number of revolutions per second is decreased. Whether the actual rate of propagation is changed would be difficult to determine. It seems evident that the wave of nerve-impulse precedes the wave of muscular contraction. The contraction of the muscle must stretch the adjacent regions, and hence stretch the region through which the nerve-impulse is passing, thus increasing the distance traveled in one revolution and decreasing the revolutions per second. When the amplitude of contraction is increased, the stretching of the nerves is increased and the revolutions per second are decreased, but whether this can account for the total decrease has not been determined. The speeding-up of the revolutions per second after the trapped wave is started is at first more abrupt and later more gradual, and is associated with both decrease in amplitude of contraction and decrease in diameter of the umbrella, due to starvation and contraction of scar-tissue.

Evidently a change in the number of revolutions per second of the trapped wave or the amplitude of the contractions would cause an error in the determination of the effect of O_2 concentration on metabolism, and in order to estimate the limits of such errors the relative metabolism of the muscle and other tissues was studied. The umbrellas of 3 cassiopeas, *a*, *b*, and *c*, of the same size (diameter = 11.5 cm.) were used (each for a series of experiments). In some experiments the rhopalia remained and the normal pulsations were generated, in others a trapped wave was induced, and in others the subumbrella was removed or merely the mesoglœa left. The apparent (but slight) metabolism of the mesoglœa was probably entirely due to a few remnants of epithelium and to bacteria, which always attack the mesoglœa when the epithelium is removed. At any rate, the metabolism of the mesoglœa is too small to be of significance. The pH was 8.2 and the O_2 per liter 4.5 c.c. at the beginning of each of the experiments.

TABLE 20.

Individual.	O_2 per hour.	Parts of umbrella used.
a	0.86 c.c.	Umbrella with 0.64 pulsation per second.
a	0.97	Umbrella with 0.64 pulsation per second.
a	0.158	Mesoglœa + exumbrella only.
b	1.12	Umbrella with trapped wave, 1.47 per second.
b	1.08	Umbrella with trapped wave, 1.47 per second.
c	0.5	Umbrella not pulsating.
c	0.6	Umbrella not pulsating.
c	0.03	Mesoglœa + bacteria.
d	0.88	Umbrella with 0.7 pulsation per second.
d	0.7	Umbrella not pulsating.

The removal of the rhopalia in the umbrellas with trapped waves or without pulsations reduced but slightly the amount of tissue. If we take the metabolism of the normal pulsating umbrella at 100, the exumbrella is about 14 and the resting subumbrella 60, with an addition of 26 for normal pulsations or 65 for trapped wave. Therefore, the neuro-muscular tissue may perform about 26 to 46 per cent of the metabolism, and changes in rate or amplitude of the contraction-wave are to be avoided as much as possible.

In subsequent experiments, the manubrium and rhopalia were removed from the cassiopea and a C-shaped cut was made through the neuro-muscular layer, about one-third the radius from the outer margin of the umbrella, and a trapped wave was induced by stimulation near the outer margin. The wave passed around the outer part of the subumbrella, and each time it passed the opening of the C it spread to the inner part, dividing into two equal waves, meeting on the far side with mutual destruction. In this way the outer part, in which the trapped wave was first induced, was made pacemaker and the result was a more permanent wave. The wave causes circulation of water against all parts of the epithelium except a small portion of the exumbrella which has a very low metabolism. If the oxygen concentration was reduced to zero, the wave stopped and metabolism ceased until oxygen was readmitted. In one experiment an umbrella was kept 7 hours at zero O_2 concentration, then 3 hours with O_2 and a trapped wave, and 7 hours at zero O_2 concentration, during which it gave out no CO_2 or other acid products affecting the pH perceptibly; 30 seconds after it was taken out of the O_2-free chamber a trapped wave was started and this constantly increased in amplitude for 10 minutes, at the end of which time the amplitude was normal.

Oxygen was removed from sea-water in various ways, with the air-pump and agitation, by boiling, and by allowing a cassiopea to remain in it until the pulsations ceased, as seen through a peep-hole in such a way that photosynthesis was practically avoided. In no case did the analysis show less than about 0.05 c.c. per liter, but that amount probably entered with the KI and $MnCl_2$ solutions and around the ground stopper of the analysis bottle. At any rate, we should consider 0.05 c.c. to be within the limit of error of the method if no correction were made for O_2 in the reagents.

The variation in metabolism after removal of the manubrium and initiation of a trapped wave is shown in table 21. The diameter of the umbrella was 11.5 cm. at the beginning of the first experiment and at the beginning of each experiment the pH was 8.2 and the O_2 per liter 4.5 c.c. It is evident that the metabolic rate may vary rapidly for 3 hours after the manubrium is removed and the trapped wave is started; therefore, in the subsequent experiments the umbrella was not placed in the respiration chamber until these 3 hours had passed. The same umbrella used in the above experiments but 21 hours after the operation was used to determine the effect of reduced oxygen concentration. The average O_2 concentration during the experiment was 1.5 c.c. per liter and the O_2 used per hour 1.03 c.c., being a decline of 20 per cent in rate of oxidation, with a reduction of the oxygen-concentration to about half its original value. This and some later experiments are as shown in table 22.

These determinations show that oxidation is reduced about 20 per cent when the O_2 concentration is reduced about 50 per cent; or the oxidation increases about 25 per cent when the O_2 concentration is increased about 100 per cent.

The above experiments show that the rate of oxidation varies with the tension of oxygen in the sea-water constantly circulated against the surface of the epithelium in which oxidation takes place. In the total absence of oxygen no measurable quantity of CO_2 or other acid products are given out, and we may infer that the metabolism is suspended. If oxygen is readmitted after a suspension of the metabolism for 7 hours, the rate of metabolism rises apparently to the normal within 10 minutes. No anaërobic processes were detected during absence of oxygen for 7 hours, but in the absence of oxygen for 16 hours, anaërobic or hydrolytic processes take place. No evidence was found to indicate that these anaërobic processes constituted the metabolism of the cassiopea. On the contrary, a great multiplication of bacteria was associated with them, and the cassiopea was partly dissolved and could not be revived by readmission of oxygen. We may assume that oxygen protects the cassiopea from the attacks of bacteria (probably anaërobes). Since the bacteria enter from the surface, it seems probable that the cassiopea might live indefinitely on so small a supply of oxygen that it is used in the superficial cells as fast as it diffuses into them, and none reaches the deeper cells. If this be true, the variation in oxygen consumption with variation of supply might be the expression of a variation in the number of cells receiving oxygen. In other words, this would be a diffusion phenomenon.

TABLE 21.

Hours after operation.	O_2 used per hour.	Revolutions of wave per second.
	c.c.	
0	1.65	1.60
2	1.55	2.00
3	1.30	2.22
4	1.30	2.22
5	1.30	2.22
20	1.30	2.22

TABLE 22.

O_2 used per hour.	
At 3.3 c.c. O_2 per liter.	At 1.5 c.c. O_2 per liter.
1.3	1.03
1.3	1.00
1.4	1.10
1.48	1.15
1.44	1.25
1.35	1.05

Another hypothesis is that the tension of oxygen at the seat of oxidation affects the rate. Warburg has shown that cell oxidation is associated with structure and that no enzyme solution has been obtained that will account for the vital oxidation of foodstuffs. If the structure or surface responsible for the oxidations is designated as the catalyst, it seems possible that the tension of oxygen in the immediate vicinity of the catalyst influences the rate. This influence of tension on rate might still be a diffusion phenomenon, since the O_2 must diffuse toward the structure-catalyst or oxidase molecule.

Owing to the excellent review of the literature on the subject of this paper by Krogh, it seems unnecessary to multiply references. Roughly speaking, and within physiological limits, animal oxidation is about doubled with 10° rise in temperature, and this is shown here to be true of *Cassiopea.* In other words, oxidation is an exponential function of the temperature as expressed in the following equation:

$$V_t = V_o \times 2^{\left(\frac{t}{10}\right)}$$

where V_t is the velocity of oxidation at $t°$ and V_o at 0°. This relation holds approximately true for a number of chemical reactions and is supposed to be due partly to change in diffusion-rate and partly to loosening of bonds in the reacting molecules and to ionization. Diffusion depends on viscosity and tension (osmotic pressure). A fall of temperature from 30° to 20° increases the viscosity of water about 25 per cent, and of a 40 per cent sucrose solution 42 per cent, and of a 3 per cent gelatine solution 1,000 per cent (von Schroeder). The increase in viscosity of gelatine, however,does not cause a proportionate decrease in diffusion. We may assume that this fall of temperature may possibly cause about 30 per cent decrease in diffusion of O_2 within the cell, due to viscosity alone. Tension is proportional to the absolute temperature. Although diffusion accounts for only about a third of the change in rate of oxidation with temperature, it is undoubtedly a factor. Since diffusion affects oxidation and concentration-gradient affects diffusion, it seems very probable that concentration of O_2 should affect oxidation, even though every cell received some oxygen.

Krogh interprets the experiments on warm-blooded animals as showing increased oxidation with increased oxygen tension, and reviews the work of Thunberg, showing the same effect to a greater degree on cold-blooded animals. Henze (1910*a*) found that the oxidation-rate of sea-anemones and annelids varies with O_2 concentration (and of nudibranchs at low concentrations) and supposed that only a varying fraction of the cells received oxygen; his tables show that the oxidation-rate of crustacea and jelly-fish fell rapidly with time, but that in one series of determinations on *Pelagia*, the oxidation-rate varied reversibly with O_2 concentration. In order to reduce the diffusion effect, he kept sea-urchin eggs agitated in sea-water and found that the oxidation-rate apparently increased about 8 per cent on doubling the O_2 concentration. Henze attributes this apparent difference in respiration to faulty technique, but since the experiments have not been repeated with improved technique, we may assume that a real difference exists.

It is interesting to compare the metabolism of *Cassiopea* with that of jelly-fish studied by Vernon. Since only the living cells metabolize, it would be an advantage to know the proportion of cellular tissue to the body-weight, but there is no data on this subject. The skeletal structure, mesoglœa, contains less organic matter than the cells, and hence the percentage of organic matter in the body is a partial indication of the cellular mass. The proportion of mesoglea increases with the size of the individual within the same species, but there can be no

TABLE 23.

Species.	Weight of individual.	Cubic centimeters O_2 per hour.	
		Per kilo body.	Per kilo organic matter.
	gm.		
Cassiopea xamachana....	100	12.5	1,040
Carmarina hastata.......	39	7.7	2,025
Cestus veneris..........	70	3.75	1,562

strict comparison between different species in this regard, and therefore the comparison is very crude. The cubic centimeters of O_2 per hour per kilogram of body-weight and per kilogram of organic matter (= dry weight − weight of salts in equal volume of sea-water) at 20° is given in table 23. The agreement is about as close as could be expected.

Vernon has compared the metabolism (per unit weight of organic matter) of jelly-fish, molluscs, tunicates, and vertebrates, and shown it to be remarkably constant. Krogh (omitting jelly-fish, but including eggs and insects) obtained the greatest differences when the total body-weights were used, but the differences probably do not exceed the differences in water-content and in muscular activity. He found the metabolism of a young dog with body-temperature lowered to 20° during the experiment to be greater than that of cold-blooded animals at the same temperature; but if we calculate the metabolism of the average dog for 20°, using a reasonably high temperature coefficient, the agreement is more satisfactory. We should not expect close agreement unless water and mineral salts and fibrous tissue are excluded from the weight and the activity of the nervous system is abolished. The chief factor in lowering the metabolism of hibernating mammals is probably the fall in body-temperature (the body-temperature may be as low as 6°).

It seems probable that the chief distinction in the calorimetry of warm-blooded and cold-blooded animals is in insulation (sensitivity to cold being the regulating factor). All warm-blooded animals are air-breathing, and air brings much oxygen and takes away little heat. The center of a cluster of bees in winter may be 40° above that of the air bathing it.

The heat-production in a 100-gram cassiopea at 30° is sufficient to raise its body-temperature 0.14° per hour above that of the surrounding water, but no such difference in temperature has been observed, because the heat generated is conducted away by the water bringing the oxygen. I found that a fish weighing 1.4 grams used 0.825 c.c. O_2 per hour at 30°, which is sufficient to raise its body-temperature about 3° per hour, but during this time it was required to breathe 400 c.c. of sea-water, even though it removed half of the oxygen from water saturated with air at this temperature. The water circulating through the gills could remove the heat generated if the body-temperature were 0.01° above that of the water. Since the fish probably removed much less than half the oxygen from the water in one passage through the gills, the body-temperature was probably much less than 0.01° above that of the water. Rogers and Lewis could detect no difference between the body-temperature of fish, salamanders, clams, and earthworms and the water in the thermostat in which they were placed, after they had been in the thermostat long enough for equilibrium. They used a thermo-couple, and one division of the galvanometer scale corresponded to 0.0042°.

It was shown that the metabolism of *Cassiopea* is proportional to its surface and not to its weight (W), but to $W^{\frac{2}{3}}$. This is due to the fact that the metabolism is confined to the living cells and that these constitute a superficial epithelium, whose thickness is about the same in cassiopeas of the range of sizes studied. We might use these results in an attempt to explain the so-called surface-law of warm-blooded animals. Dreyer, Ray, and Walker have shown that the blood-volume and cross-sections of the aorta and trachea are proportional to $W^{\frac{2}{3}}$ (or surface). If animals were of the same shape (internally as well as externally), the cross-sections of all organs would be proportional to $W^{\frac{2}{3}}$, but the blood-volume would be proportional to the weight (W). If the blood-volume is proportional to $W^{\frac{2}{3}}$, the whole circulatory system would be nearly proportional to $W^{\frac{2}{3}}$, and owing to the close relation between the lungs and the blood the volume of the lungs would be nearly proportional to $W^{\frac{2}{3}}$. The volume of the skin may be proportional to $W^{\frac{2}{3}}$ and the volume of the wall of the alimentary tract nearly so. The nerve, muscle, and glandular tissues are excitable, and hence their metabolism must vary. Variable components may be excluded from basal metabolism by definition, but can not all be eliminated in making measurements. Only the skeleton can be said to have a constant metabolism proportional to W, and since the red bone-marrow produces blood (erythrocytes) and this is proportional to the surface, the metabolism of some of the bones may be nearly proportional to the surface. Benedict has shown that great variations from the surface law exist, and hence it may be only accidental.

The fact that the excitable tissues metabolize more per unit weight in small animals than in large (*i. e.*, proportional to a smaller power of W than unity) is true, not only for warm-blooded animals, but also for cold-blooded animals, to which the teleological principle of the surface law (in relation to heat regulation) does not apply. It seems possible, however, that the chief conditions necessary for the evolution of temperature-regulation in animals were: (1) air breathing; (2) large body size; (3) sensitivity to low temperatures; (4) variation of activity of excitable tissues with size; and (5) epithelial type of architecture, 4 and 5 being more characteristic of cold-blooded animals.

It is hoped that the fact that metabolism varies with O_2 tension may explain the increase in metabolism in certain types of acidosis. Lusk and Richie have shown that certain amino acids have a specific dynamic action and Benedict asserts that it has been demonstrated that there is a distinct increase in the basal metabolism with the acidosis resulting from the ingestion of a carbohydrate-free diet. It is not assumed that amino acids dissociate enough H ions or neutralize enough base to cause acidosis, and whether there is any relation between the phenomena discussed by Lusk and by Benedict may be open to question, but it seems clear that increased metabolism may accompany acidosis.

Acidosis develops in the acclimatization to high altitudes and persists some time after the descent, during which time the subject feels more vigorous (Schneider). During acidosis the ventilation of the lungs is increased to such an extent that the alveolar air is increased, and therefore the O_2 in the blood-plasma is increased and that in the hemoglobin is increased if it is not already saturated. Increased respiratory movements tend to increase the heart-rate and hence the circulation, with better oxygen transfer to the tissues and increased oxidation. This effect may be small, and may be too small to account for the total increase in metabolism, but is worthy of consideration. Rasmussen has shown mild acidosis in case of the hibernating woodchuck with very much lowered O_2 tension of venous blood, but evidently the sensitivity of the respiratory center falls during hibernation.

It was shown by Warburg that the metabolism of sea-urchin eggs may be stimulated, but these are to be considered irritable cells in the same class with muscle. It may be that the metabolism of all cells can be stimulated, but they are not equally sensitive. Changes in hydrogen-ion concentration of the sea-water effective on sea-urchin eggs are not nearly so effective on the cells of *Cassiopea* (possibly because the pH of the sea-water changes before reaching the cells). Benedict claims that the basal metabolism of the human body may be stimulated, but it is difficult to estimate basal metabolism on account of the unavoidable activity of some excitable organs. Cutting the nerves is of no advantage, since Langley and Itagski showed that the oxygen consumption of denervated muscle is much greater than that of normal resting muscle. What cells are stimulated in Benedict's experiments is not clear. He adds that changes in the pulse-rate accompany changes in the basal metabolism, and thus admits that irritable cells of the complex neuro-muscular organization of the body are concerned in the phenomenon. If the respiratory or vasomotor centers are concerned in the increased metabolism, O_2 tension in the tissues may be a factor. Verzár showed that resting muscle and active heart use more oxygen when there is more in the blood. The submaxillary gland used oxygen at the same rate in all experiments, but suffocation of the animal occurred when the oxygen tension in the gland was about 40 mm. Rasmussen (see also Dubois) has shown that the oxygen concentration of the venous blood of marmots (woodchucks) is lowered during hibernation, although the oxygen capacity of the blood remains the same. This shows that the O_2 tension of the blood is lowered both by fall in concentration and temperature, and this may be a factor in the lowered metabolism. The O_2 concentration of the arterial blood is not reduced, but the rate of blood-flow is very much reduced, so that less oxygen is carried to the tissues.

ON THE CHEMICAL PRECIPITATION OF $CaCO_3$ IN SEA-WATER.*

The question of the solubility of calcite and aragonite in sea-water is a matter of interest in relation to the geology of limestone and dolomite. Murray and Hjort maintain that sea-water is so complicated a mixture that the solubility of $CaCO_3$ can not be calculated with certainty (from the law of mass action), but that the experiments of Anderson and of Cohen and Raben show that sea-water is saturated with calcite. They add (p. 181) that dolomite is less soluble than calcite in carbonated water. Their book summarizes observations showing that calcium carbonate is precipitated in shallow tropical waters, but that even shells are dissolved in the red-clay bottoms of the depths.

Mayer (1916) placed pieces of *Cassis* shell in sea-water for more than a year and found that they maintained their weight within about 0.1 per cent. The precipitation of $CaCO_3$ at Tortugas was studied by T. Wayland Vaughan, R. B. Dole, and G. H. Drew. Drew observed that a denitrifying bacillus, *Pseudomonas calcis*, obtained from the sea-water was capable of changing calcium nitrate to calcium carbonate in culture media and supposed a similar process to occur in sea-water. Since Vaughan has observed that calcium carbonate is constantly precipitating at Tortugas, Drew's hypothesis necessitates the presence of an appreciable amount of nitrates or nitrites, and I have attempted to determine them.

A half liter of sea-water was boiled in an all-glass still and the distillate collected in a series of 25 c.c. Nessler's tubes; another series of Nessler's tubes were filled with a graded series of concentrations of ammonium chloride; 1 c.c. of Nessler's reagent was added to each tube and agitated; after 15 minutes the tubes were compared colorimetrically and the ammonia recovered from the sea-water was estimated; after no more ammonia could be distilled from the sea-water, amalgamated aluminium shavings were introduced into the still and the distillation process was repeated. The ammonia recovered was formed by reduction of nitrates and nitrites. Duplicate analyses gave less than 0.01 mg. of nitrogen per liter as ammonia and less than 0.01 mg. nitrogen per liter as nitrates and nitrites. Raben found more than 10 times these quantities in North Sea water (Murray and Hjort, p. 368). Evidently *Pseudomonas calcis* and other organisms have almost completely removed the fixed nitrogen from Tortugas sea-water. The effect of this probably explains the scarcity of life in the vicinity of Tortugas as compared with colder seas (law of minimum). There is, however, a constant renewal of fixed nitrogen from the atmosphere, from the decay of organisms, and probably from water rising from the depths of the ocean. If *Pseudomonas calcis* is an important agent in the precipitation of $CaCO_3$, its action is evidently more intense in places where calcium salts, nitrates, and nitrites are carried from the land into the sea.

---

*The content of this section was published as a separate paper in the Proc. Nat. Acad. Sci., 1917, III, 612.

That calcium carbonate is withdrawn from surface waters of the sea is shown by chemical analyses. Dittmar found an average of 0.44 per cent less calcium in surface waters than in deeper waters. This is true, notwithstanding the fact that calcium carbonate is constantly being added to the surface waters. The drainage of the land contains an excess of calcium carbonate and flows out on the surface of the sea, where the water evaporates, leaving the excess of $CaCO_3$ in the sea-water. The action of organisms in building calcareous structures may account for a large part of the depletion of surface waters, but the precipitation of calcareous mud at Tortugas has been observed by Vaughan.

The analysis of the calcium content of sea-water requires double precipitation and filtration for separation from magnesium, and hence large samples and great care are required for accuracy. Theoretically, however, we may detect differences in calcium content by titration. Dittmar showed that sea-water is remarkably constant in composition, except for H_2O, calcium, and gases. The water content is inversely proportional to Cl and the gases may be eliminated by boiling after the addition of enough acid to decompose the carbonates. If we disregard carbonic acid, there is an excess of bases in sea-water—*i. e.*, the sum of the base equivalents is greater than the sum of acid equivalents. Since calcium is added to or taken from sea-water in the form of $CaCO_3$, any change in the calcium content causes an equivalent change in the excess base or alkaline reserve, as it is called by chemists. The alkaline reserve may be titrated while boiling the sea-water to eliminate CO_2. The exact value of the titration depends on the indicator used and the exact color of the indicator that is taken as the end-point; hence only those titrations done in the same manner can be strictly compared (see method described in first part of this paper). The alkaline reserve per liter was recorded and some titrations were made at 20° and others at 30°, but the errors due to change in volume of the sea-water is within the limits of accuracy of the method. If the sea-water is diluted with rain-water, the alkaline reserve will be lowered, but this error may be compensated by dividing by Cl. In other words, a change in the quotient of the alkaline reserve by Cl indicates a gain or loss of $CaCO_3$.

TABLE 24.

Sea-water from—	Alkaline reserve.	Cl.	$\frac{\text{Alk. res.}}{\text{Cl}} \times 10{,}000$
San Diego	0.00235	18.7	1.257
Wood's Hole	.00240	17.7	1.356
Gulf Stream, off Miami	.0025	19.9	1.257
Gulf Stream, off Tortugas	.0025	19.9	1.257
Average, Tortugas, June and July	.00247	20.0	1.235
Average, Key West, June and July	.00237	20.0	1.185

Table 24 indicates that some $CaCO_3$ has been removed from Tortugas sea-water, as compared with other sea-water, and to a greater extent from Key West sea-water. In other words, the precipitation observed by Vaughan is not due to a greater amount of calcium in Tortugas or Key West sea-water, but to local conditions which cause the precipitate to form.

According to the law of mass-action, in a saturated solution of $CaCO_3$, in sea-water at constant temperature, salinity, etc.,

$$[Ca^{\cdot\cdot}] \times [CO_3''] = \text{a constant}$$

Not all of the calcium is, however, in the form of $CaCO_3$ and $Ca^{\cdot\cdot}$, for some is undissociated $CaCl_2$, $CaSO_4$, $Ca(OH)_2$, and $CaHCO_3$. The chlorides and sulphates are constant, but $[CaHCO_3]$ and $[Ca(OH)_2]$ change with the total CO_2 content of the sea-water. But I have shown (McClendon, 1917*b*) that if the alkaline reserve remains constant the total CO_2 of the sea-water (within limits found in nature) varies inversely with the pH ($= -\log. H^{\cdot}$ concentration). Hence the determination of the pH may be substituted for that of the total CO_2.

The determinations I have made of the water of the Pacific and North Atlantic showed the pH to vary from about 8.1 to 8.25 and those of Dr. A. G. Mayer in the Pacific showed only a little wider range (table 11). Earlier observations at Tortugas gave the same range, but my more extended observations in this summer of 1917 show the inadequacy of a few determinations. The pH is influenced by plant and animal life and rises at Tortugas to 8.35 during the day over well-lighted bottoms rich in vegetation, and falls to 8.18 during the night. It may be said, therefore, that conditions in shallow water over eel-grass or other seaweed or corals (with symbiotic algæ) favor the precipitation of $CaCO_3$.[1]

The question arises whether the occasional high pH of Tortugas sea-water is sufficient to explain the precipitation of $CaCO_3$, or whether nuclei for the separation of the solid phase are necessary. A large amount of $CaCl_2$ may be added to sea-water without causing a precipitation. If the pH is increased by the addition of NaOH, the result depends on the speed at which the alkali is added. If the NaOH is added suddenly in the form of a strong solution, colloidal precipitation membranes form about the drops and if the membranes are broken by shaking or stirring, a great mass of $Mg(OH)_2$ is included in the precipitate. If a very dilute solution of NaOH is added very slowly, $CaCO_3$ possibly contaminated with $Mg(OH)_2$ is precipitated. The exact pH at which precipitation first occurs can not be determined by this method, as the first precipitation occurs before the solutions are mixed and the crystals thus formed serve as nuclei for further precipitation. If Tortugas sea-water is kept in glass bottles, precipitation occurs on the glass while the pH of the water is within the natural limits, but the pH at the glass surface is higher, due to solution of glass.

---

[1] It would be interesting to know whether corals and calcareous algæ deposit as much $CaCO_3$ in the dark as in the light. Corals from deep water are smaller, more fragile, and deposit less $CaCO_3$ than those of shallow water, but the same is true of animals without symbiotic algæ. The deposition is, however, related to the pH, since Palitzsch has shown that the pH decreases with depth.

Although the pH at which precipitation would occur without nuclei for the separation of the solid phase may be practically impossible to determine, the final equilibrium with an abundance of nuclei is not a difficult problem. Calcite and aragonite crystals to serve as nuclei were produced by the methods of Johnston, Merwin, and Williamson. The crystals were examined under the microscope and tested with cobalt-nitrate solution. These observations, together with the mode of preparation, leave little doubt that the crystals actually were calcite and aragonite. Under the microscope an occasional calcite crystal could be found among the aragonite crystals, but the number was not sufficient to affect the cobalt-nitrate test. These calcite crystals seemingly grew slightly during the experiments, but apparently no new ones were formed. To determine the equilibria, crystals were mechanically stirred or shaken with sea-water in "nonsol" flasks, 6 to 14 hours at 30°; then the pH and alkaline reserve were determined.

The results of agitating 100 grams of calcite crystals with a liter of sea-water until equilibrium was approximately reached also appear in figure 7. The alkaline reserve is measured on the ordinate and the pH on the abscissa. This shows that sea-water of the surface of the oceans of the whole world is supersaturated in respect to calcite. We may therefore conclude that suitable nuclei for the precipitation of calcite are absent or deficient in number. The solubility of crystals varies inversely with their size, but after they have attained sufficient size to be readily examined with low powers of the microscope further increase has an unappreciable effect on solubility. But such crystals, if present, would rapidly gravitate to the bottom of the sea; hence the absence of nuclei for precipitation of calcite is what one might expect.

Aragonite is said to be about 10 per cent more soluble than calcite, but no difference in the point of equilibrium of the two substances with sea-water was detected in these experiments (fig. 8). This may be explained by the fact that a few calcite crystals were mixed with the aragonite, equilibrium was only approximated, and there were slight errors in the determinations.

During the rough weather, white calcareous mud is stirred with the sea-water at Key West and to a lesser extent at Tortugas, and it was thought possible that the mud granules might form nuclei for precipitation and explain the low alkaline reserve at Tortugas and lower alkaline reserve at Key West. On agitating white calcareous mud, dredged from the bottom, with sea-water, no definite equilibrium was reached, even at the end of 4 days. If the alkaline reserve was first lowered by removal of some $CaCO_3$, it remained lower than if shaken with calcite, and if normal sea-water was used the alkaline reserve remained higher than with calcite. It was thought possible that the grains were covered with an impenetrable film of organic matter; so some mud was dried and powdered in a mortar to break the pieces and form fresh surfaces, but similar results were obtained with this.

If mud was mixed with an equal weight of calcite, the results were the same as with pure calcite. This mud and calcite on standing in sea-water for 30 days had not all changed to calcite. All these facts

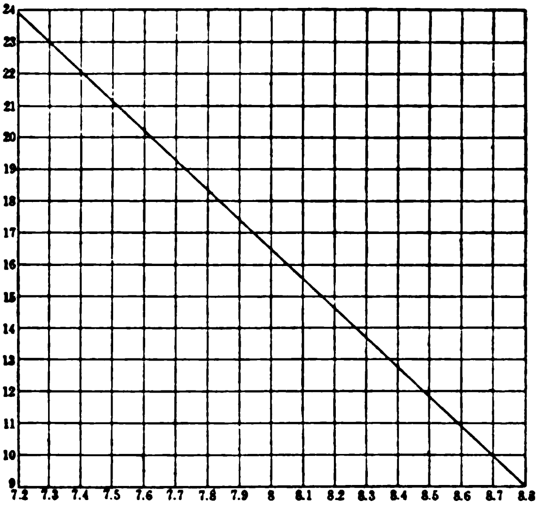


FIG. 7.—Equilibrium of calcite with Tortugas sea-water. The pH is marked on the abscissa and the alkaline reserve × 10,000 on the ordinate.

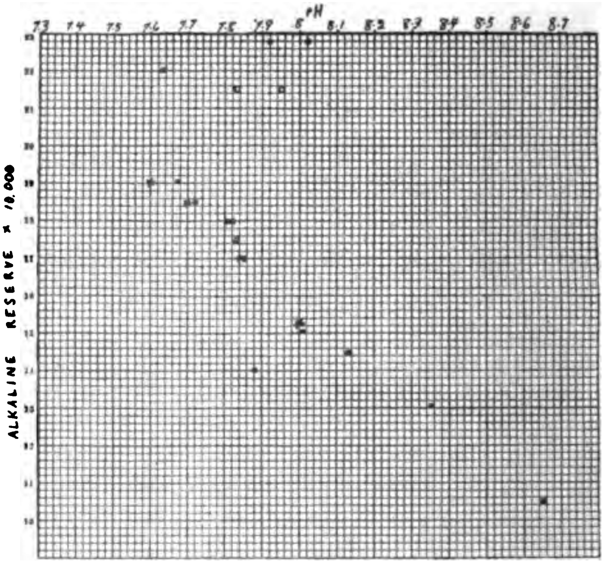


FIG. 8.—The equilibrium of $CaCO_3$ with Tortugas sea-water. Calcite is denoted by black rhombs, aragonite by rosettes, calcareous mud by black circles (no equilibrium), mud and calcite by black dumb-bells, and pulverised coral by white circles.

tend to show that the particles in the mud are in some way retarded or prevented from getting into equilibrium with the water.

In order to be sure of clean surfaces of natural calcareous substances, a specimen of coral, *Mæandra clivosa*, was ground and powdered in a mortar and agitated with sea-water in the same manner as in previous experiments. The results were similar to those with mud (fig. 8).

There seems to be a more soluble form of calcium carbonate (the $\mu CaCO_3$ of Johnston), but since it can not be obtained in a pure state no attempt was made to prepare it. One experiment, however, was made with a precipitate of $CaCO_3$ that appeared as spherical grains under the microscope. It was agitated for 12 hours with sea-water and the alkaline reserve was 0.0022 at pH 7.95. No further experiments were made to determine whether equilibrium had been approximated.

These experiments clearly show that the surface water of the sea is a supersaturated solution of $CaCO_3$; and it is only necessary to introduce calcite crystals in order to cause considerable precipitation of this substance. Precipitation goes on in the bodies of organisms in the surface waters of all seas. The precipitation observed by Vaughan at Tortugas is very finely divided, but whether it was formed in the bodies of minute organisms, which subsequently died, has not been determined. Such particles might slowly grow, since the agitation of them with sea-water was found to take a trace of $CaCO_3$ out of the water. Small crystals have been seen in the bodies of *Protista* and, whether they are $CaCO_3$ or not, they might form nuclei for the precipitation of $CaCO_3$ if released into the sea-water.

TABLE 25.

Sea-water.	pH.	Total CO_2.	Alkaline reserve.	Calculated loss of CO_2.	
				From total CO_2.	From alkaline reserve.
Before agitation with calcite..	8.2	44.5	0.0025	6	6.72
After agitation with calcite...	7.67	38.5	.0019	...	....
Before agitation with calcite..	8.25	43.8	.00250	6.8	7.27
After agitation with calcite...	7.72	37	.00185	...	....

In some experiments in liter flasks of resistance glass, filled to the neck (and hence admitting but slight loss of CO_2), the pH and alkaline reserve were determined immediately before and after agitation with calcite. The loss of CO_2 from the sea-water was estimated in two ways: (1) the remaining CO_2 was calculated from the pH and alkaline reserve; (2) the loss of $CaCO_3$ was calculated from change in alkaline reserve.

In table 25, the agreement is very striking, in view of the probable error in determination of pH and the liability to loss of CO_2 from the water surface in the neck of the flask, agitated by the rotary stirrer. If the pH of sea-water should be maintained (by the action of plants) at 8.2 while it was agitated with calcite crystals, the loss of $CaCO_3$ would be about 0.001 N, or 0.0005 M, or 0.1 gram per liter. This would cause a deposit of 10 kg. per square meter of bottom in water 100 meters deep. This would cause a lowering of the total calcium content of Tortugas sea-water by about 4.5 per cent.

The actual precipitation of $CaCO_3$ was most noticeable in the Marquesas lagoon. At 4 p. m., July 30, the pH was 8.46 and there was a precipitate of $CaCO_3$ coming down in the water and incrusting the eel-grass.

BIBLIOGRAPHY.

For an extensive bibliography see Krogh, or McClendon, 1917 (*a*).

Benedict, F. G. 1915. Jour. Biol. Chem., xx, p. 263.
Blackman, F. F. 1905. Annals of Botany, xix, p. 281.
———, and A. M. Smith. 1910. Proc. Roy. Soc. (B), lxxxiii, p. 389.
Burrows, M. T. 1917. Amer. Jour. Physiol., xliii, p. 13.
Carlson, A. J. 1911. Amer. Jour. Physiol., xxvii, p. 323.
Cary, L. R. 1917. Carnegie Inst. Wash. Pub. No. 251, p. 121.
Dittmar, W. Voyage of H. M. S. *Challenger*, Physics and Chemistry, vol. 1.
Dole, R. B. 1914. Carnegie Inst. Wash. Pub. No. 182.
Drew, G. H. 1914. Carnegie Inst. Wash. Pub. No. 182.
Dreyer, G., W. Ray, and E. W. A. Walker. 1912. Proc. Roy. Soc., lxxxvi, pp. 39, 56.
Dubois, R. 1894. Compt. Rend. Soc. Biol. (ser. 10), i, p. 821.
Haberlandt. 1917. Physiological Plant Anatomy (Trans. by Drummond).
Hatai, S. 1917. Carnegie Inst. Wash. Pub. No. 251, p. 95.
Heilbrunn, L. V. 1915. Science, xlii, p. 615.
Henze, M. 1910(*a*). Biochem. Z., xxvi, p. 266.
———. 1910(*b*). Abderhalden's Biochemische Arbeitsmethoden, iii, p. 164.
Johansen, A. C., and A. Krogh. 1914. Consul Permanent Int. pour l'Exploration de la Mer, Pub. de Circonstance No. 68, p. 30.
Johnston, J., H. E. Merwin, and E. D. Williamson. 1916. Amer. Jour. Sci., xli, p. 473.
Krogh, A. 1916. The Respiratory Exchange in Animals and Man.
Langley, J. N., and M. J. Itagaki. 1917. Physiol., li, p. 202.
Loeb, J., and H. Wasteneys. 1912. Biochem. Z., xl, p. 277.
Lusk, G., and J. A. Richie. 1912. Jour. Biol. Chem., xiii, p. 27.
Mayer, A. G. 1906. Carnegie Inst. Wash. Pub. No. 47.
———. 1908. Carnegie Inst. Wash. Pub. No. 102, p. 115.
———. 1914. Carnegie Inst. Wash. Pub. No. 183, p. 55.
———. 1916. Proc. Nat. Acad. Sci., ii, p. 28.
———. 1917. Carnegie Inst. Wash. Pub. No. 251, p. 1.
McEwen, G. F. 1916. Univ. California Pub. (Zool.), xv, p. 255.
McClendon, J. F. 1917(*a*). Phys. Chem. Vital Phenomena.
———. 1917(*b*). Jour. Biol. Chem., xxx, p. 265.
———, and P. H. Mitchell. 1912. Jour. Biol. Chem., x, p. 459.
Murray, Sir John, and J. Hjort. 1912. The Depths of the Ocean, p. 178.
Palitzsch, S. 1911(*a*). Biochem. Zeitsch, xxxvii, p. 116.
———. 1912(*b*). Measurement of the Hydrogen-Ion Concentration in Sea-Water. Danish Oceanographical Expeditions, 1908–1910, vol. i, Hydrography, p. 237 Copenhagen.
Rasmussen, A. T. 1915. Amer. Jour. Physiol., xxxix, p. 20.
———. 1916. Amer. Jour. Physiol., xli, p. 162.
Rogers, C. G., and E. M. Lewis. 1916. Biol. Bull., xxxi, p. 1.
Taylor, H. F. 1917. U. S. Bureau of Fisheries, Doc. No. 848.
Vaughan, T. Wayland. 1914. Carnegie Inst. Wash. Pub. No. 182.
Vernon, H. M. 1895. Jour. Physiol., xix, p. 18.
Verzár, F. 1912. Jour. Physiol., xlv, p. 39.
von Schroeder, P. 1903. Z. physik. Chem., xlv, p. 75.

Tortugas
v 11 ^

This book may be ke[illegible]
[illegible]

DATE DUE

89036529105

b89036529105a

BIOLOGY

GY LIBRARY

89036529105

b89036529105a